Dentin bonding—Variables related to the clinical situation and the substrate treatment

Jorge Perdigão*

Department of Restorative Sciences, Division of Operative Dentistry, University of Minnesota School of Dentistry, 515 SE Delaware St., 8-450 Moos Tower, Minneapolis, MN 55455, USA

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Abstract
The wetness of dentin surfaces, the presence of pulpal pressure, and the thickness of dentin are extremely important variables during bonding procedures, especially when testing bond strength of adhesive materials in vitro with the intention of simulating in vivo conditions. The ultimate goal of a bonded restoration is to attain an intimate adaptation of the restorative material with the dental substrate. This task is difficult to achieve as the bonding process is different for enamel and for dentin—dentin is more humid and more organic than enamel. While enamel is predominantly mineral, dentin contains a significant amount of water and organic material, mainly type I collagen. This humid and organic nature of dentin makes this hard tissue very challenging to bond to. Several other substrate-related variables may affect the clinical outcome of bonded restorations. Bonding to caries-affected dentin is hampered by its lower hardness and presence of mineral deposits in the tubules. Non-curious cervical areas contain hypermineralized dentin and denatured collagen, which is not the ideal combination for a bonding substrate. Physiological transparent root dentin forms without trauma or caries lesion as a natural part of aging. Similar to the transparent dentin observed underneath caries lesions, the tubule lumina become filled with mineral from passive chemical precipitation, making resin hybridization difficult. An increase in number of tubules with depth and, consequently, increase in dentin wetness, make bonding to deeper dentin more difficult than to superficial dentin. While the application of acidic agents open the pathway for the diffusion of monomers into the collagen network, it also facilitates the outward seepage of tubular fluid from the pulp to the dentin surface, deteriorating the bonding for some of the current adhesives. Some dentin desensitizers have shown some promise as they can block dental tubules to treat and prevent sensitivity and simultaneously blocking the tubular fluid from flowing to the surface. A new approach to stop the degradation of dentin–resin interfaces is the use of MMP inhibitors. Although still in an early phase of in vitro and clinical research, this method is promising.

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1. Introduction to the review

Dentin tubules run continuously from the dentin–enamel junction (DEJ) to the pulp in coronal dentin, and from the cementum–dentin junction (CEJ) to the pulp canal in the root. Pashley (1996) described dentin as a porous biologic compos-
Fig. 1 – Caries lesion under the FESEM showing: (A) partially demineralized dentin in the transition to caries-affected dentin; (B) tubules are occluded with mineral deposits in the caries-affected area.

ite made up of apatite crystal filler particles in a collagen matrix [1]. Other authors have described dentin as a complex biological structure that forms a continuous fiber-reinforced composite, with the intertubular dentin making up the matrix and the tubule lumens with their associated cuffs of peritubular dentin forming the cylindrical fiber reinforcement [2]. Marshall et al. [3] stated that the various structural components and properties of dentin could directly affect the adhesive bond. Biological and clinical factors such as dentin permeability, pulpal fluid flow, sclerotic and carious dentin can also affect dentin bonding [4–7].

During the development of the human tooth the dentin that is secreted until the completion of root formation is identified as primary dentin and encompasses the circumpulpal dentin matrix. This primary dentin forms at a rate of approximately 4 μm per day [8] although the rate of dentin formation is slower in the root than in the crown of the tooth. Physiological secondary dentin is secreted after completion of root formation. The appearance of the matrix of primary dentin indicates a regular, tubular structure. While secondary dentin may be less tubular, it has the same morphological features of primary dentin.

The morphological and physical variations in human dentin make it a difficult substrate for the achievement of durable bonds between adhesive resin and dentin. The bonding mechanism depends on the penetration of the primer and adhesive resin into the conditioned dentin surface in order to create micromechanical interlocking with the dentin collagen. This mechanical entanglement between resin monomers and dentin components has been named hybrid layer [9] or resin–dentin interdiffusion zone [10].

2. Literature

2.1. Caries-affected dentin and tertiary dentin

The most common pathological challenge to dentin is dental caries. According to the pioneer work by Dr. Fusayama’s research team, carious dentin consists of a superficial first layer and a deeper second layer [11–13]. The outer layer was characterized as highly decalcified, physiologically unrecalcifiable, fuchsinstainable, showing degenerated collagen fibers with virtual disappearance of cross-links indicating irreversible denaturation of collagen. The inner layer was depicted as intermediatey decalcified, physiologically recalcifiable, fuchsins-stainable, with expanded odontoblastic processes, sound collagen fibers, and apatite crystals bound to the fibers. Changes in the cross-linkage of collagen in this inner layer showed cross-links partly shifting to precursors. In spite of a great deal of work that has been accomplished on the biochemical principle of carious dentin staining, it remains unclear how staining is related to ultra-structural features of various caries lesion zones [14]. Level of bacteria found in dye-stained DEJ specimens was no higher than the specimens that did not stain with the same dye [15]. Stainable dentin may not be always infected but the absence of stain does not ensure bacterial elimination [15,16]. In fact, dyes may not specifically stain the irreversibly damaged collagen found in infected dentin [17] and may even stain dentin in non-curious teeth [18]. These findings raise questions about the validity of studies on bonding to caries-affected dentin in
which the caries-infected dentin is removed with the guidance of dyes.

Changes that will occur in dentin structure in response to the carious process take place either within the dentin itself, or on its pulpal aspect. The pulp responds to the carious process either by completely blocking the lumen of the dentinal tubule, or by a decrease in tubule diameter, to assist in the prevention of further permeation of bacteria and toxic materials towards the pulp. Stanley et al. [19] found that the pulp-dentinal complex responds to external injuries with dentin sclerosis, dead tracts, or reparative dentin. The presence of reactive dentin sclerosis does not prevent the formation of reparative dentin, since both seem to occur in response to the same stimuli. Mendis and Darling in 1979 reported that the amount of peritubular dentin in the transparent zone under the SEM was similar to that in normal non-caries dentin and that the remaining lumen was occluded by a rod-like plug of granular structure not continuous with peritubular dentin [20]. It has been reported that these deposits in the dentinal tubules are β-tricalcium phosphate (also called whitlockite) [21,22], which is less soluble than hydroxyapatite.

In fact, continuous deposition of mineral within the lumina of the tubules underneath a caries process will lead ultimately to their obliteration and the formation of sclerosis (Fig. 1). This type of sclerotic material differs from physiological sclerosis in that its formation can be triggered by the carious process, restorative procedures, or attrition and, in these cases, its formation is restricted to the site of the affected area [8,14]. The tubule occlusion is most apparent in transparent or sclerotic dentin, leading to its transparent optical appearance [14] and higher mineral contents than normal dentin [20].

The tissue that is deposited on the pulpal aspect in response to dental caries is called reactionary tertiary dentin. Unlike secondary dentin, which is physiological and forms throughout the vital life of the tooth, the formation of tertiary dentin is localized in the pulp chamber wall corresponding to the area of the affected site. Furthermore, tertiary dentin is divided into reactionary dentin, formed by the surviving odontoblasts, and reparative dentin, which is formed by newly differentiated odontoblast-like or odontoblastoid cells [8,23]. The reactionary form of tertiary dentin frequently has a tubular continuity with the physiological secondary dentin and is secreted by primary odontoblasts [23]. Fig. 2 shows reactionary tertiary dentin from attrition in a lower incisor, which, in this case, does not have tubular continuity with secondary dentin. The reparative dentin bridge form of tertiary dentin does not have a tubular continuity with the physiological secondary dentin matrix. Reparative tertiary dentin is secreted by odontoblastoid cells, which have replaced the irreversibly injured odontoblasts at the site of pulp exposure [23].

While bonding to tertiary dentin might only occur in deep cavity preparations, caries-affected dentin is a clinically relevant substrate in daily practice. Much of our understanding on dental bonding has been performed on sound, healthy dentin (‘laboratory’ dentin). This is not the substrate generally encountered in clinical practice, since dentists frequently bond to caries-affected dentin to replace carious tissues. Caries-affected dentin has lower hardness than normal dentin. Although the transparent zone is usually thought to be harder than unaffected dentin due to occlusions of the dentin tubules with mineral in the transparent zone, it has been reported that the transparent layer in caries-affected dentin can be softer than unaffected dentin [24]. Using a nanoindentation technique, Marshall et al. [25] reported that the mean elastic modulus (18.2 GPa) and nanohardness (0.8 GPa) of transparent intertubular dentin were very slightly, but...
significantly lower than those of the intertubular dentin of unaffected dentin (20.6 and 1.0 GPa, respectively). However, within a given lesion, there were frequently no significant differences, suggesting that the intertubular dentin was often unchanged in the transparent zone. For transparent dentin, the mean elastic modulus of peritubular dentin was 36.1 GPa, which was not significantly higher than the mean value for the mineral within the tubules of 34.5 GPa, but both were significantly less than the modulus for normal peritubular dentin (38.7 GPa). A significant correlation was found between dentin hardness and bond strength, however, dentin mechanical properties are not the only factors responsible for lower bond strength to caries-affected dentin. A micro-Raman spectroscopy investigation suggested some structural or chemical alterations in caries-affected dentin [26]. The mineral phosphate and carbonate content decreased in the caries-affected region of dentin when compared with unaltered dentin [26]. The secondary structure of collagen in the caries-affected dentin also appeared to be slightly altered by the caries process. These results were corroborated by Suppa et al. [27] who found that the distribution of intact collagen fibrils and proteoglycans in the caries-affected dentin was significantly lower than in normal hard dentin. Reductions in antigenicity from the organic matrix of sclerotic dentin under caries lesions raise concern about the potential of intrafibrillar remineralization.

Bond strengths to caries-affected dentin are typically lower than those obtained in normal unaffected dentin, regardless of the type of adhesive used [28–31]. A comparison of three different adhesive strategies (one-step self-etch, two-step self-etch, and two-step etch-and-rinse) resulted in lower bond strength to caries-affected dentin than to normal dentin for all of the adhesives [29]. The lower bond strengths obtained in caries-affected dentin may be due to the lower tensile strength and lower hardness of the altered substrate. Clinically, this may not be a problem, since such lesions are normally surrounded by normal dentin or enamel [30]. Hybrid layers in caries-affected dentin are usually thicker but more porous than those in sound dentin [30,31]. Hybrid layers were less than 1 μm thick in normal dentin, but that they were between 6 and 8 μm thick in caries-affected dentin [32]. An atypical interface was observed in caries-infected dentin, in which carious bacteria within disorganized non-banded collagen fibrils were apparently embedded by the adhesive, Clearfil Liner Bond 2V (Kuraray). The hybrid layer in caries-infected dentin was 30–60 μm thick [32]. The monomers infiltrated the depth of demineralized unaltered dentin, and the penetration gradually declined across the interface; however, the penetration of adhesive monomers was irregular and decreased across the depth of demineralization in the caries-affected dentin [26]. As a result of the deposition of mineral in the lumina of the tubules, resin infiltration into dentinal tubules of caries-affected dentin is also hampered by the presence of mineral [31]. Using FTIR, Spencer et al. [33] described the interface with caries-affected dentin as a poorly mineralized structure readily penetrated by the acid etchant. The substantial area of demineralization in the caries-affected dentin indicates a porous area. The degree of conversion of the adhesive (Single Bond, 3 M ESPE) that penetrated the demineralized dentin in the caries-affected dentin specimens was lower than in the normal dentin specimens [33]. Tay et al. [34] applied a one-step self-etch adhesive on transparent carious dentin containing occluded dentinal tubules, with or without pulpal pressure. After silver impregnation, they observed specimens under TEM. Although caries-affected dentin was highly porous, silver particles did not infiltrate the adhesive layer when tubules were occluded. Conversely, water-treeing and water-droplet formation might be eliminated during bonding to occluded transparent carious dentin.

Bonding to caries-affected dentin may depend on the specific composition of adhesives. One study compared the bond strengths of ScotchBond Multi-Purpose (3 M ESPE) with and without the polyalkenoic component in the primer. Removal of the polyalkenoic acid from the primer lowered the bond strength of ScotchBond Multi-Purpose to caries-affected dentin [35], which suggests that the residual calcium in caries-affected dentin may be crucial to establish ionic bonding with the polyalkenoate in the primer. Using Clearfil SE Bond (Kuraray), Nakajima et al. [36] reported that hydrostatic pulpal pressure significantly reduced the bond strength to normal dentin after 1 month of water storage, but did not affect the bond strength to caries-affected dentin. The presence of mineral casts in the tubules may have prevented water seepage to the area of the bonded interface. Additionally, the higher mineral contents of the transparent area of caries-affected dentin may have resulted in stronger chemical bonding to the MDP molecule in Clearfil SE Bond, which has been shown to bond chemically to hydroxyapatite [37].

Another study reported that when One-Step (Bisco Inc.), an acetone-based etch-and-rinse adhesive, was used on caries-affected dentin etched with 10% phosphoric acid, it resulted in lower tensile bond strengths compared to normal dentin. However, this difference disappeared when 32% phosphoric acid was used instead of 10%. For the ethanol-based etch-and-rinse adhesive Single Bond (3 M ESPE) the bonds made to caries-affected dentin were always lower than bonds to normal dentin regardless of the concentration of the phosphoric acid gel [38]. Different results were obtained in another study [39] with Single Bond (3 M ESPE). The authors compared two polyalkenoic acid copolymer-containing adhesives applied to normal versus caries-affected dentin. While the one-step self-etch adhesive Adper Prompt L-Pop (3 M ESPE) resulted in significantly higher bond strengths to normal dentin than to caries-affected dentin, the bond strengths of the two-step etch-and-rinse adhesive Single Bond to both normal and caries-affected dentin were not significantly different. Additionally, the hybrid layers produced by both adhesive systems were thicker for caries-affected dentin.

In addition to specific composition of dentin adhesives, different caries dentin removal methods may also influence adhesion to caries-affected dentin [40]. In this study, the authors used three removal methods: round steel bur in a slow-speed handpiece; Er:YAG laser; or 600-grit SiC abrasive paper. The specimens were bonded with Clearfil Protect Bond (Kuraray) or with OptiBond Solo Plus Total-Etch (Kerr) and tested in microtensile mode. Clearfil Protect Bond resulted in significantly lower bond strength compared to OptiBond Solo Plus Total-Etch after caries dentin removal with a round bur. However, the opposite was observed for specimens treated...
with abrasive paper. For laser-treated dentin, there were no significant differences between the two groups.

2.2. Sclerotic dentin in non-carious cervical lesions (NCCL)

There is now evidence of the multifactorial etiology of NCCL [41,42]. Occlusal stresses may play a role in the pathogenesis of these tooth defects [43,44]. Sclerotic dentin is common in areas where dentin is exposed (NCCL and attrition areas), being a more complex substrate than unaltered dentin due to different ultra-structural layers [45]. As stated by Mendis and Darling (1979), “a translucent zone is observed under carious dentin, but not under toothbrush abrasion lesions” [20]. The same authors reported that, “in abrasion, SEM of longitudinal fractures showed the surface was homogeneous with completely occluded tubules. Beneath this surface layer, most tubules were empty.”

One of the most common clinical symptoms from NCCL is tooth sensitivity. Sensitivity is usually associated with tubular patency. Most tubules are occluded with mineral casts in insensitive transparent sclerotic dentin regions [46]. Gwinnett and Jendresen [47] observed that the dentin surface in cervical erosion lesions appeared smooth with evidence of intratubular deposits. Acid conditioning with 50% phosphoric acid for 60s opened up tubules in ‘eroded’ dentin, many of which had become occluded by intratubular deposits. The penetration of resin (N-phenyl glycine-glycidyl methacrylate, Cervident, S.S. White) was limited by the presence of these deposits following acid conditioning. When applied to acid-conditioned, ‘eroded’ dentin, resin penetrated approximately 30 μm compared to more than 100 μm into the tubules of acid-etched normal dentin [47].

The matrix of the 10–20 μm thick surface hypermineralized layer in sclerotic dentin of NCCL is composed of denatured collagen with bacteria colonizing the surface of the lesion [45]. Collagen may be denatured as a result of acids and bacterial by-products in the surface layer. Additionally, larger hydroxyapatite crystallites are observed in the hypermineralized surface layer as compared to sclerotic dentin. The transition from denatured collagen to intact collagen with cross-condensation is evident at the base of the hypermineralized layer [45]. Crystalline deposits that obliterate the tubules etch more slowly than the other dentin components. The fact that intertubular sclerotic dentin from NCCL etches differently than normal root dentin may explain the difficulties in restoring such lesions with current bonding procedures [48].

Irrespective of the use of a total-etch or a self-etch technique, bonding to sclerotic dentin in NCCL has resulted in compromised bonding [49,50]. In one study [50], Clearfil Liner Bond 2V, a self-etching adhesive, was applied to sclerotic cervical dentin with or without 40% phosphoric acid etching. Regardless of the dentin treatment, bond strengths to unaffected dentin were consistently higher than those made to sclerotic dentin. However, phosphoric acid etching improved bonding to the most cervical part of the lesion. Phosphoric acid was not able to etch below the hypermineralized surface layer nor was it able to dissolve the residual sclerotic casts in the tubules. Tay et al. [51] reported that bond strengths to sclerotic dentin using a self-etching primer (Clearfil Liner Bond II Σ, Kuraray) were 20% lower than those obtained in sound cervical dentin regardless of the location of the bonding substrate inside the lesion. These same authors used EDS to quantify the relative concentration of calcium and phosphorus in NCCL. While the Ca/P ratio was similar in the surface hypermineralized layer (1.67) compared to the underlying sclerotic dentin (1.71), that ratio was 1.38 in the sclerotic casts within the tubules. Additionally, they found a small percentage of Mg (4.57 wt%) in the composition of these sclerotic casts, confirming that they are composed of whitlockite as other authors had reported. The same authors [51] also listed four factors that may influence the decrease in bond strengths to sclerotic dentin in NCCL: (1) the presence of a hybridized microbial matrix together with entrapped bacteria; (2) inability of the self-etching primer/adhesive to dissolve and penetrate the thick surface hypermineralized region; (3) presence of a layer of denatured collagen at the base of the hypermineralized zone; (4) presence of residual sclerotic casts that obliterate the tubules and prevent tag formation.

In spite of the lower bond strengths obtained when the substrate is sclerotic dentin from NCCL, clinical studies have not totally corroborated the in vitro findings. Van Dijken [52] found that the differences between the sclerotic and non-sclerotic and the roughened and non-roughened lesions were not significant. One might argue that the bonding agent used in this study was a resin-modified glass-ionomer material (Fuji Bond LC, GC Co.), which does not depend on mechanical interlocking with collagen or formation of resin tags. Nonetheless, in another study by the same author [53], 144 NCCL (98 with sclerotic dentin, 46 non-sclerotic) were restored with one of three adhesives Clearfil Liner Bond 2 (Kuraray), One Coat Bond (Coltène/Whaledent), and Prompt L-Pop (3 M ESPE). The cumulative loss rates of the materials in sclerotic lesions (15.7%) versus non-sclerotic lesions (14.0%) were not significantly different. Diamond bur-roughened lesions showed a loss rate of 14.5%, while for the non-roughened lesions the frequency was 14.8%.

According to the 2001 ADA guidelines for enamel and dentin adhesive materials [54], resin-based adhesives gain “provisional acceptance” at 6 months if their retention rate in non-carious cervical lesions (NCCL) is at least 95% without mechanical retention features. Full acceptance requires a 90% retention rate at 18 months. Due to the fact the NCCL are the recommended and most frequently used substrate for clinical tests of dentin adhesives, the degree of dentin sclerosis has been ranked from 1 to 4 to standardize this particular variable. When no dentin sclerosis is visibly present, NCCL are given a score of 1 for sclerosis [55,56]. The score 4 is attributed to NCCL in which dentin is dark yellow or brownish with glassy aspect [55,56].

Clinical studies that categorize the degree of sclerosis of NCCL are not abundant. In one recent study [56], no differences in retention rates were found for a mild all-in-one adhesive at 3 years when applied to NCCL with a sclerosis score of 1–2 versus when it was applied to NCCL with a sclerosis score of 3–4. However, the marginal adaptation at 3 years was slightly, but not significantly better for the group with sclerosis scale 1–2 (81% αopa ratings) compared to the group with sclerosis scale 3–4 (70% αopa ratings). There were three missing restorations
in NCCL with a sclerosis scale equal to 4 in this study, but they corresponded to the group in which the all-in-one adhesive was used after total-etch [56].

Regarding the adhesion strategy (i.e., self-etch versus etch-and-rinse), there have been conflicting reports pertaining to the common belief that etch-and-rinse adhesives bond more effectively to dentin in NCCL in clinical situations than self-etch adhesives [56–59]. Clearfil SE Bond (Kuraray), an MDP-based self-etch adhesive, results in excellent retention in NCCL at 5 years [59]. For this specific adhesive, chemical bonding to the hypermineralized substrate in NCCL may supersede the need for separate dentin etching.

### 2.3. Substrate changes with aging

The tubule volume in coronal dentin was calculated as 10%, without great difference between old and young teeth [60]. Overall dentin thickness increases at a rate of approximately 6.5 μm per year for coronal dentin [61]. The thickness of mantle dentin and globular dentin of aged teeth are less than that of young teeth. The hardness and modulus of elasticity of aged dentin are higher than those of young dentin at the mantle dentin level. The reactionary dentin in aged teeth and newly developed secondary dentin in young teeth have lower modulus of elasticity and hardness than those of other circumpulpal dentin [62].

As mentioned above, secondary dentin is deposited over the entire inner circumpulpal surface throughout the life of the tooth. Unlike secondary dentin, which is physiological, the formation of tertiary dentin is localized in the pulp chamber wall corresponding to the area of the affected site, usually a caries process. There is also a form of physiological sclerotic or transparent dentin that starts in the root and increases with age linearly. The pattern of distribution is similar in all teeth. Physiological transparent dentin is first formed in the apical dentin adjacent to the cementum and extends coronally and towards the root canal, with increasing age [63]. Physiological transparent root dentin, as distinguished from pathological transparency subjacent to caries, appears to form without trauma or caries lesion as a natural part of aging. Similarly to the transparent dentin observed under a caries lesion, transparent dentin from aging occurs when the tubule lumina become filled with mineral from passive chemical precipitation [63,64], decreasing the amount of light scatter, therefore the translucent nature. It is not clear whether the increased mineralization associated with transparency is entirely a result of the filling of the tubule lumina, or whether there are any additional alterations in the mineralization of the intertubular dentin matrix. As tubules become filled with mineral in transparent dentin, the mineral concentration, as measured by X-ray computed microtomography, is significantly higher in transparent dentin and the fracture toughness of dentin decreases by 20% [64]. The elastic modulus is unchanged in physiological transparent dentin; however, transparent dentin, unlike normal dentin, exhibits almost no yielding before failure [64]. Other authors [65] have reported that fatigue strength of young dentin (17–30 years) is greater than that of older dentin (50–80 years) and that aging results in increase in both the rate of damage initiation and propagation in dentin.

As a result of the reduction in tubule diameter there is also a decrease in dentin permeability. Teeth of subjects over 50 years contain less water than teeth 10–20 years of age, becoming more brittle. With increasing patient age, in both crown and root aspects of teeth, dentinal thickness increases, while the density of odontoblasts and pulp fibroblasts decreases [61]. The degree of age-related changes in teeth is asymmetrical, with decreases of cell density in the root being more evident than in the crown. At all ages pulp cell densities, including odontoblasts, within the crown are greater than in the root. Decreases in pulp cell density may reduce pulp repair activity after restorative treatments, although increases in dentinal thickness may aid with pulp protection [61].

Tagami et al. [66] compared the tensile bond strengths of four dentin bonding systems using proximal dentin of premolars extracted from young (9–21 years) and old (42–64 years) patients. For each adhesive, there were no differences in bond strengths between young and older teeth. An adhesive failure tended to occur at the interface between the bonding resin and the resin-impregnated layer regardless of substrate age. Brackett et al. [67] measured the microtensile bond strengths of an etch-and-rinse resin adhesive (Prime & Bond NT, Dentsply) and a resin-modified glass-ionomer adhesive (Fuji Bond LC, GC Co.) on teeth known to have originated from subjects over 60 years of age. They used as control teeth originating from young subjects. No significant differences were observed between the young and aged teeth for any comparison. SEM evaluation of the etched dentinal surfaces demonstrated less depth of decalcification in the intertubular areas of aged dentin, but there was no observable difference within the tubules of young and aged dentin. In another study [68], authors used extracted molars from three age groups (20–25, 35–40, and 50–55 years) to evaluate the microtensile dentin bond strengths of two self-etching materials. For one of them (ABF or Clearfil Protect Bond, Kuraray), bond strengths were higher in the 35–40 years age group.

### 2.4. Tooth region and remaining dentin thickness (RDT)

The area occupied by tubule lumina at the DEJ is approximately 1% of the total surface area at the DEJ and 2% at the pulp [1,60]. Since this area is occupied by dentinal fluid, which is 95% water, these areas are also approximately equal to the tubular water content of these areas. That is, the water content of dentin near the DEJ is about 1% (volume), while that of dentin near the pulp is about 22% (Fig. 3). This difference in intrinsic moisture has been deemed responsible for the differences in bond strengths between superficial and deep dentin. Superficial dentin normally results in higher composite-dentin bond strengths than deep dentin [69–73]. For example, Suzuki and Finger (1988) reported that bond strengths decreased 30–40% in deep dentin for three dentin adhesives [73], whereas Nakamichi et al. [69] reported a 50% decrease in bond strength from superficial to deep dentin in bovine teeth. These differences tend to diminish when the smear layer is left intact, but lower bond strengths occur in deep dentin when the smear layer is removed [74]. As bonding systems became more hydrophilic, the sensitivity of bond strengths to dentin depth has decreased [75].
The orientation of the dentinal tubules may also influence dentin bond strengths. Bond strengths of four all-in-one adhesives to dentin with different tubule orientations showed no significant differences in microshear bond strengths regardless of dentin depth (superficial or deep) or dentin tubule orientation (perpendicular or parallel/oblique) [76]. However, for the adhesives Clearfil SE Bond (Kuraray), OptiBond Solo Plus SE (Kerr), and Clearfil S5 Bond (Kuraray) there were significantly lower bond shear strengths to deep dentin with a tubule orientation perpendicular to the bonding surface [76]. Another study [77] tested Clearfil SE Bond and OptiBond Solo Plus varying the dentin location (occlusal or cervical), dentin depth (superficial or deep) and dentinal tubule orientation (perpendicular or parallel) to the bonding surface. No statistically significant differences were found in shear bond strengths based on dentin location. In contrast to the results of the previous study [76], Clearfil SE Bond resulted in higher shear bond strengths to deep dentin specimens bonded perpendicular to the tubules compared to those that were bonded parallel to the tubules, while the opposite was found for OptiBond Solo Plus. In the case of superficial dentin, there were no differences between the two materials for the different tubule orientations. In this particular study, shear bond strengths were affected by dentin depth, orientation of the tubule and the adhesive material, but not by location of the dentin (occlusal or cervical). On the contrary, Phrukkanon et al. [78] reported that bond strengths of Single Bond (3 M ESPE) and an experimental self-etching primer were not affected by tubule orientation.

The orientation of the dentinal tubules has a profound effect on the formation of the hybrid layer associated with etch-and-rinse adhesives. In areas with perpendicular tubule orientation, the layer was 3.2 μm thick, showing solid 27.2 μm long resin tags, and a network of tubule anastomoses. In areas with parallel tubule orientation the layer was significantly thinner (1.3 μm) and resin tags were absent [79].

Other regional variables may influence dentin bonding. Occlusal dentin tends to give lower bond strengths than buccal dentin [80]. However, in this case depth may have been a confounding variable, as noted by the authors. There is also a greater regional variability of dentin wetness in occlusal dentin than in proximal or buccal dentin [75].

2.5. **Smear layer**

Residual organic and inorganic components form a “smear layer” of debris on the surface whenever dentin is prepared with a bur or other instrument [81,82]. This structure has a very low inherent bonding of 5 MPa to the dentin substrate [83]. The smear layer fills the orifices of dentin tubules forming “smear plugs,” and decreases dentin permeability by up to 86% [84]. Sub-micron porosity of the smear layer still allows for diffusion of dentinal fluid [83]. The composition of the smear layer is basically hydroxyapatite and altered denatured collagen. Composition changes with depth to reflect the composition of dentin in different areas of the tooth [83]. This altered collagen may even acquire a gelatinized consistency as a result of the friction and heat created by the preparation procedure [85].

Current adhesives are classified according to the way they interact with the smear layer. This classification results in two bonding strategies and four types of adhesives:
1. Etch-and-rinse (or total-etch) adhesives include a separate acid-etching step, usually with 30–40% phosphoric acid applied simultaneously on enamel and dentin to remove the smear layer and superficial hydroxyapatite.
2. Self-etch adhesives do not rely on a separate acid-etching step; they include an acidic monomer solution that is not rinsed off, making the smear layer permeable without removing it completely.
   b. One-step self-etch adhesives (one solution or all-in-one).

The removal of the smear layer and smear plugs with acidic solutions results in an increase of the fluid flow onto the exposed dentin surface. This fluid can interfere with adhesion [81], because hydrophobic resins do not adhere to hydrophilic substrates even if resin tags are formed in the dentin tubules [86].

One factor that might interfere with the demineralization potential of a self-etch adhesive is the instrument used to create the smear layer. Dentin surfaces ground with diamond burs tended to present more compact smear layers than those ground with SiC papers. Smear layer denseness, more so than thickness, may compromise bonding efficacy of adhesives, especially of self-etch systems [87]. Some studies reported low dentin bond strengths over thick dentin smear layers [88,89], while others reported no influence [90,91]. This can be partially explained by differences in roughness, smear layer thickness (ranging from 0.9 to 2.6 mm), density, and attachment to the underlying tooth structure, which is dependent on the way the smear layer is created [92].

Mowery et al. found that 600-grit SiC paper resulted in lower bond strength of the second-generation adhesive Scotchbond Multi-Purpose (3 M) to dentin than did 60 grit SiC [93]. Finger, on the other hand, did not find significant differences for several adhesives in function of the roughness of the surface preparation method [94]. Tay and Pashley studied the aggressiveness of three self-etching adhesives in penetrating dentin smear layers of different thickness (600- or 60-grit SiC paper) using fractured dentin as control [95]. They used an aggressive adhesive, Prompt L-Pop (3 M ESPE), a moderate adhesive, NRCP/Prime&Bond 2.1 (Dentsply), and a mild adhesive, Clearfil SE Bond (Kuraray). For the mild self-etch adhesive the smear layer and smear plugs were retained as part of the hybridized complex. For the moderate self-etch adhesive, the smear layer and smear plugs were completely dissolved in dentin with thin smear layers, but partially retained with thick smear layers. For the aggressive self-etch adhesive, the smear layer and smear plugs were completely dissolved and formed hybrid layers. For the aggressive self-etch adhesive, the smear layer thickness had no effect on resin-dentin bond strength of Clearfil SE Bond (Kuraray), Optibond Solo Plus Self-Etch, Tyrian/One Step Plus, Single Bond, and Scotch-Bond Multi-Purpose Plus. Kenshima et al. [96] confirmed the results obtained by Tay and Pashley [95], observed that thick smear layers were not totally removed by the mild self-etch primer Clearfil SE Bond (Kuraray). Thicker hybrid layers were observed for the strong self-etch adhesive Tyrian Self Priming Etchant + One Step Plus (Bisco) and for the etch-and-rinse adhesive Scotchbond Multi-Purpose (3 M ESPE).

One study [92] attempted to correlate the smear layer created with a carbide bur group with that created with 320-grit abrasive paper. Human dentin was abraded with 0.05 μm alumina, 240-, 320- or 600-grit abrasive paper, # 245 carbide, # 250.9 F diamond or # 250.9 C diamond burs. Shear bond strengths decreased with increasing coarseness of the abrasive for the self-etching adhesive (Clearfil SE Bond, Kuraray). The higher bond strengths and thin smear layer of the carbide bur group, suggests its use when self-etch materials are used in vivo. The 320-grit abrasive paper yielded results closer to that of the carbide bur and its use is recommended in vitro when using Clearfil SE Bond.

The mode of application (i.e., with or without agitation) may also result in different degrees of penetration of mild self-etch adhesives through smear layers [98]. With passive application, self-etch adhesives diffused through thick smear layers and formed thin hybrid layers in intact dentin. With continuous agitation, smear layers were completely dispersed or dissolved, and thicker hybrid layers with upstanding collagen fibrils were observed.

### 2.6. Dentin permeability and pulpal pressure

Dentin tubules are slightly tapered, with the wider portion oriented toward the pulp. Dentin permeability increases almost logarithmically with cavity depth; such an increase is attributed to the vast differences in the sizes and numbers of dentinal tubules between superficial and deep dentin [60]. According to the hydrodynamic theory [99], once dentin is exposed, external stimuli cause fluid shifts across dentin, which activate pulpal nerves and cause pain. Transdental permeability is also responsible for the constant wetness of exposed dentin surfaces due to the outward fluid movement from the pulp [100].

The smear layer presents a limitation to the diffusion to fluid movement, as it decreases dentin permeability by occluding the tubule orifices [101]. Increased dentin permeability, in terms of fluid flux and hydraulic conductance, has been reported after the surface was modified with phosphoric acid [102,103], citric acid [104], or polyacrylic acid [105]. Acids remove the smear layer, increasing the dentin permeability. Smear layers created with 600-grit silicon carbide abrasive paper reduce the hydraulic conductance of extracted human teeth to 20–24% of the maximum values obtained after acid-etched dentin [102].

Dentin permeability results in dentin surface wetness, which influences the quality of the adhesive-dentin interface and may decrease the bond strength between resins and dentin [100]. Etch-and-rinse adhesives result in higher micro-permeability compared to self-etch adhesives [106]. The hybrid layer was always 100% infiltrated by pulpal fluid when an etch-and-rinse adhesive was used. However, pulpal pressure had no effect on enamel sealing [106]. Other studies have reported that a simulated pulpal pressure decreases the dentin bond strengths of resin-modified glass-ionomer and...
etch-and-rinse adhesive materials [107,108]. Under simulated pulpal pressure, CLSM observations revealed a distinctly shallower penetration of etch-and-rinse adhesives into the dentin tubules compared to the dentin treated without intrapulpal pressure [109]. However, the use of hydrophobic bonding agents applied on acid-etched dentin saturated with ethanol reverses the fluid conductance to the level obtained with the presence of the smear layer [110].

Other studies have reported that the simulated positive pulpal pressure during bonding application does not seem to affect the in vitro performance of self-etch adhesives significantly [111,112]. All-in-one adhesives are more susceptible to pulpal pressure than two-step self-etch adhesives [113] as a result of the application of a hydrophilic resin layer in the latter. Sauro et al. applied simulated pulpal pressure during the build-up procedure instead of during the bonding application, using self-etch adhesives [113]. While Clearfil Protect Bond (Kuraray), a two-step self-etch adhesive, exhibited the lowest permeability and fewest numbers of fluid droplets over the surface of the bonded dentin, the all-in-one adhesives G-Bond (GC Co.) and Clearfil S² Bond (Kuraray) and One Up Bond F (Tokuyama) were more permeable than Clearfil Protect Bond. The application of pulpal pressure significantly reduced bond strength.

Application errors in the clinical sequence may also influence dentin permeability. For OptiBond FL (Kerr), a three-step etch-and-rinse adhesive, the highest mean percentage of permeability reduction was observed in the group where the adhesive was applied as per manufacturer’s instructions [114]. Clinically, most dental restorations are performed under local anesthesia, which may change the permeability of dentin. Permeability data from in vitro studies must be interpreted with caution. In clinical conditions there is an outward fluid flow across exposed dentin in response to the low, but positive pulpal tissue pressure (14.1 ± 2.5 cm H₂O for an RDT of 1.06 ± 0.04 mm in premolars) [115]. Pitt-Ford et al. [116] demonstrated that 2% lidocaine without vasoconstrictor produced no reduction in pulpal blood flow. On the other hand, Beveridge and Brown [117] reported that lidocaine without vasoconstrictor produced a transient increase in pulpal pressure, which is consistent with the vasodilating properties of lidocaine. Kim et al. [118] demonstrated that lidocaine with 1:100,000 epinephrine lowered pulpal blood flow in dogs 72% after infiltration and 67% after block anesthesia. Olgart and Gazedius [119] reported that infiltration of the vasoconstrictors, such as epinephrine or felypressin, decreased pulpal blood flow.

2.7. Treatment prior to bonding—desensitizers

According to the hydrodynamic theory [99], dentin sensitivity is caused by rapid fluid shifts across dentin that activate intrapulpal nerves and cause pain. In light of this theory, blocking dentinal tubules should prevent fluid shifts and prevent dentin sensitivity. Pashley et al. [120] measured the direction and magnitude of fluid shifts across dentin in dentin surfaces in response to several hydrodynamic stimuli: air blast, 56 °C water, 2 °C water, tactile and osmotic. In acid-etched superficial dentin, the greatest fluid flow was obtained for hot water followed by cold water. The osmotic and the tactile stimuli caused the least fluid flow across dentin.

Treating acid-etched dentin with 3% potassium-hydrogen oxalate re-occluded the dentinal tubular openings with insoluble crystals. The authors suggested that this treatment might be useful as a dentin cavity liner to re-close dentinal tubules after dentin is acid-etched [101]. This technique was later used with three adhesives, Single Bond (3 M ESPE), One-Up Bond F (Tokuyama), and AdheSE (Ivoclar Vivadent). These adhesives were unable to eliminate the fluid flow through dentin. The application of 3% potassium oxalate prior to the bonding procedures was suggested as an effective technique in reducing the dentin permeability, regardless of the adhesive used [121]. However, in order to be effective, the oxalate-based material must be applied after dentin is etched. In one study [122], dentin was treated with four oxalate desensitizers before or after acid-etching, and bonded with an acetone-containing two-step etch-and-rinse adhesive. Microtensile bond strengths were significantly lower, compared with the control, when oxalates were used before the specimens were acid-etched. When oxalates were used after acid-etching, although the microtensile bond strength was similar to the controls [122]. Additionally, ‘silhouettes’ of subsurface crystals were observed inside the dentinal tubules under the TEM. The adhesive layer did not display any signs of nanoleakage when the oxalates were applied after acid-etching. Other studies have not found favorable results with oxalates applied with a dentin adhesive [123]. The application of an oxalate gel or an oxalate in liquid form with Single Bond (3 M ESPE), an etch-and-rinse two-step adhesive, produced significantly lower long-term microtensile bond strengths and enhanced nanoleakage after 3 months of simulated pulpal pressure.

Gluutaraldehyde is used as a fixative that cross-links proteins [124]. Glutaraldehyde-based substances, such as Gluma Desensitizer (5% glutaraldehyde and 35% HEMA in water, Heraeus Kulzer), have been used in dentistry to reduce dental hypersensitivity under restorations and on exposed sensitive dentin [125,126]. The mechanism behind the reduction in dentin sensitivity is that these glutaraldehyde-containing desensitizers reduce dentin permeability. One study evaluated in vitro the dentin permeability of HEMA-based desensitizing in dogs for 1 week, 1 month and 3 months. At the end of the 3-month period, Gluma Desensitizer had the lowest permeability value, therefore provided a longer lasting tubule-occluding effect [127]. Another study used human molar dentin slices to compare in vitro the efficacy of five resin-based desensitizing agents, including Gluma Desensitizer, at reducing human dentin permeability. All the desensitizing agents resulted in a large decrease in dentin permeability [128]. The same glutaraldehyde-based desensitizing agent has been suggested as a re-wetting agent on etched dentin to help prevent post-operative sensitivity under posterior com-
posite restorations [129]. In spite of the favorable in vitro bond strengths [129,130], clinically it is not very effective for this specific application [131], as the operative technique may be more relevant than the use of any desensitizer [131].

The pulpal toxicity of desensitizing agents must be questioned especially considering that some of these materials are applied on patent dentinal tubules. In one study, the toxicity was measured on L 929 fibroblasts. None of the materials resulted in toxicity to the fibroblast culture [128]. Additionally, glutaraldehyde-containing materials disinfect dentin in vitro [132].

The effect of glutaraldehyde- and HEMA-based desensitizers on collagen is also a relevant issue. Ritter et al. [133] demonstrated that the treatment of demineralized dentin with 37% phosphoric acid did not significantly disrupt or dissociate the major cross-links of dentin collagen. According to the authors, in a clinical setting where phosphoric acid is applied to mineralized collagen for less than 1 min, the stability and structure of collagen are most likely unaffected. When Gluma Desensitizer was applied to acid-etched dentin it affected dentin collagen amino acid and cross-link composition—reduction of free lysine (Lys) and hydroxylysine (Hyl) residues, as well as a decrease in the levels of collagen reducible cross-links [133].

2.8. Treatment prior to bonding—chlorhexidine

Chlorhexidine was first used in dentin bonding as a dentin disinfectant. The research problem was whether chlorhexidine decreased dentin bond strengths when used as a dentin disinfectant [134]. Scanning electron microscopy revealed that the chlorhexidine solution deposited debris on the dentin surface and within the tubules of etched dentin [134], but chlorhexidine had no significant effect on the shear bond strengths of composite to dentin using the All-Bond 2 adhesive (Bisco Inc.).

Besides being a commonly used antibacterial rinse used in Periodontology, chlorhexidine is now used in dental adhesion as a protease inhibitor [135]. Metalloproteinases (MMP) are a class of zinc- and calcium-dependent endopeptidases capable of degrading all extracellular matrix components [136–138]. The exposed collagen fibrils may be vulnerable to degradation by endogenous MMP after acid-etching [136]. Collagenolytic and gelatinolytic activities found in partially demineralized dentin [135] imply the existence of MMP in human dentin, which contains gelatinases MMP-2 and -9, collagenase (MMP-8), and enamelysin MMP-20 [136–138]. These enzymes are trapped within the mineralized dentin matrix during odontogenesis [137,138]. There are, nevertheless, still some unanswered questions on this subject. For example, the role of MMP-2 in dentin bonding is not clear, as its immunoreactivity is localized preferably in predentin and around the DEJ in teeth from subjects 12–30 years [139]. Consequently, its availability in middle dentin is questionable. Additionally, chlorhexidine may damage stem cells when used for root canal irrigation [140].

Dentin collagenolytic and gelatinolytic activities can be overcome by protease inhibitors [135], indicating that MMP inhibition might preserve the integrity of the hybrid layer. In fact, the in vivo application of chlorhexidine improves the integrity of the hybrid layer [141]. When chlorhexidine was applied in vitro, the integrity of the hybrid layer and the magnitude of bond strengths were preserved in aged dentin–resin interfaces [142]. When phosphoric acid is applied without the subsequent application of chlorhexidine, it does not inhibit the collagenolytic activity of mineralized dentin, while the use of chlorhexidine after acid-etching, even in very low concentrations, strongly inhibits that activity. The drawback that might be associated with the use of chlorhexidine is the potential discoloration associated with its use [143].

2.9. Storage conditions/storage time

Pashley et al. found no differences in bond strengths between in vivo versus in vitro in a dog model for extraction times of 30 min, 1 day, 1 week, and 1 month [144]. Additionally, post-extraction time had no effect on hydraulic conductance through dentin [145]. Aquilino et al. stored teeth for 3 months in 0.9% aqueous NaCl, 0.05% saturated solution of thymol, or distilled water. After applying Scotchbond (3 M ESPE), they found no differences in bond strengths [146]. Mitchem and Gronas found no difference in dentin bond strength between 30 min and 2 years post-extraction time [72].

Another storage method that researchers have used is cryopreservation. A study compared freshly extracted teeth, cryopreserved teeth, or teeth stored in 0.5% chloramine at 4°C. Authors concluded that refrigeration at 4°C in 0.5% chloramine for 48 days or longer may cause an increase in microleakage while cryopreservation for 13 weeks or short-term refrigeration did not affect the microleakage [147].

More recently, Lee et al. [148] stored extracted teeth at 37°C for 60 days in distilled water, 0.9% NaCl, 0.5% chloramine-T, 5.25% NaOCl, 2% glutaraldehyde, or 10% formalin. A subset of 10 specimens from all groups was autoclaved, while a subset of 10 specimens from all groups (except formalin) was kept in 0.5% NaOCl for 14 days. Storage in NaOCl resulted in lower bond strengths while sterilization with the autoclave negatively affected the bond strength of specimens stored initially in distilled water or 10% formalin. Sterilization with formalin alone had no significant effect on bond strengths, therefore authors recommended 10% formalin as storage medium for in vivo studies.

According to the ISO technical specification 11405 [149], “ideally the bond strengths should be measured immediately post-extraction, but this is not generally feasible. It appears that most changes occur in the initial days or weeks after extraction. Therefore, teeth 1 month, but not more than 6 months, after extraction should be used. NOTE: teeth that have been extracted for longer than 6 months could undergo degenerative changes in dentinal protein. The teeth should then be placed in distilled water (grade 3, ISO 3696) or in a 0.5% chloramine-T trihydrate bacteriostatic/bacteriocidal solution for a maximum of 1 week, and thereafter stored in distilled water either in a refrigerator (i.e., nominal 4°C), or frozen at below –5°C. To minimize deterioration, the storage medium should be replaced periodically. It is essential that no other chemical agents be used, as they may be absorbed by, and alter, tooth substance.”
REFERENCES


