Japanese Dental Science Review (2011) **47**, 31—42

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journal homepage: www.elsevier.com/locate/jdsr

Review article

New strategy to create "Super Dentin" using adhesive technology: Reinforcement of adhesive—dentin interface and protection of tooth structures

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Received 11 September 2009; received in revised form 7 March 2010; accepted 12 April 2010

KEYWORDS
Acid—base resistant zone;
Dentin bonding;
Hybrid layer;
Secondary caries;
Super Dentin;
Adhesion

Summary Dentin bonding systems have been dramatically simplified and improved during the recent decades. Monomer penetration into dentin and its polymerization in situ creates a hybrid layer, which is essential to obtain good bonding to dentin. Moreover, the presence of an acid—base resistant zone below the hybrid layer has been documented with self-etching adhesive systems in an artificial secondary caries attack. When ultrastructure of the acid—base resistant zone is assessed by SEM and TEM observations, formation of the acid—base resistant zone is considered to be due to the monomer penetration potential and fluoride release in the adhesive systems. Natural dentin has a limited potential to resist an acid attack of secondary caries; however, the acid—base resistant zone does not purely consist of dentin in morphology, it is rather a combination of dentin and the adjacent hybrid layer. Therefore, the reinforced dentin has been called "Super Dentin" bearing the ability to prevent primary and secondary caries. Prospectively, the great potential of adhesive technology in creation of the "Super Dentin" would lead to the development of new materials for mechanical, chemical and biological protection of the dental structures.

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1882-7616
doi:10.1016/j.jdsr.2010.04.002

Contents

1. Introduction

Dentin bonding systems have been dramatically simplified and improved during the past decades.

Monomer penetration into dentin and its polymerization in situ creates a hybrid layer, which is essential to obtain good bonding to dentin [1]. Theoretically, the hybrid layer can provide marginal sealing of the cavity and resist against acid challenge to prevent secondary caries [2]. However, it was reported that none of the adhesives currently available could completely eliminate nanoleakage along the dentin-restorative interface [3].

The concept of minimal cavity preparation has become widely accepted for the placement of direct composite restorations by using an adhesive system [4]. On the other hand, recurrent caries is still considered to be one of the major reasons for failure of resin composite restorations [5]. Several methods have been developed for laboratory evaluation of secondary caries, assessing demineralized lesions and inhibition zones of dentin after acid challenge. These include polarized light microscopy [6], microhardness [7], microradiography [8], confocal laser-scanning microscopy and the X-ray analytical microscope [9]. However, each of these methods has its own limitations, making it difficult to obtain detailed information at the interface between cavity and adhesive restoration.

Scanning electron microscopy (SEM) has been shown to be a powerful device for analyzing the ultrastructure at the dentin/adhesive interface. Using an SEM, Tsuchiya et al. foremost observed artificial secondary caries inhibition around restorations bonded to bovine root dentin [10]. A new zone, the so-called "acid—base resistant zone" (ABRZ), was found beneath the hybrid layer in SEM observation, which was completely different from the inhibition zone formed due to release of fluoride from materials such as a glass-ionomer cement; in fact, the acid—base resistant zone was formed in spite of the adhesive being fluoride-free [10,11]. Ultrastructural assessment of the ABRZ has considerably advanced as the specimen preparation procedures for SEM and TEM observations of ABRZ are established.

This paper has reviewed the previous studies on assessment of ultrastructure of the ABRZ at the adhesive—dentin interface by SEM and TEM observations. Also, the mechanism of the ABRZ formation and a new concept of "Super Dentin" have been discussed.

2. Classification of dentin bonding systems

Several classifications of dentin bonding systems have been suggested in the past and in scientific literature. However, no consensus concerning terminology has been reached yet [12]. According to the concept and mechanism of the adhesive systems, recent dentin bonding systems can be classified into two main categories: self-etching primer systems and acid-etching systems. The category of self-etching primer systems is further divided into two sub-categories: two-step self-etching primer systems and one-step self-etching primer systems, including the so-called "all-in-one adhesive systems". A two-step self-etching primer system is composed of a self-etching primer and an adhesive. The self-etching primer contains one or several acidic monomers in their components that can condition and prime dentin surface simultaneously. In the one-step adhesive systems, the roles of the self-etching primer and the adhesive are combined into one application step. On the other hand, the category of acid-etching systems contains conventional acid-etching systems, three-step etching/priming/bonding systems and two-step etch and rinse systems, which can be recognized by an initial etching step. Current acid-etching systems usually use 30—40% phosphoric acid, which removes the smear layer, while concurrently demineralizing dentin over a depth of 3—5 μm [12]. Therefore, phosphoric acid etching is much more aggressive in demineralization of the dentin surface, compared to the self-etching primers.

3. Visualization of the hybrid layer

As mentioned earlier, it is essential to create a hybrid layer at the resin—dentin interface in order to obtain proper adhesion. The hybrid layer is created by penetration and polymerization of adhesive monomers, after removal and/or modification of the smear layer and superficial demineralization of the dentin [1]. Previously, the hybrid layer between dentin and an adhesive was attempted to be visualized under the SEM, using chemical and/or mechanical modifications of the interface.

In the chemical approach, the hybrid layer was subjected to the combination of an acidic solution, such as hydrochloric acid [13] and phosphoric acid [14], and sodium hypochlorite. An acidic solution demineralizes the inorganic component of

dentin structure and removes hydroxyapatite, leaving organic dentin components. Sodium hypochlorite was used to remove the demineralized collagen to enable a clear visualization of the hybrid layer. Therefore, by definition, the hybrid layer is characterized as a layer resisting against acid challenge [1].

As for the mechanical approach, argon-ion beam etching has been used to clearly reveal the hybrid layer at the resin–dentin interface [15]. Roughening of the hybrid layer through argon-ion beam etching seems to be caused by selective removal of the impregnated resin component in demineralized dentin. As a result of the edge effect of the etched surface, this layer was clearly distinct in the secondary electron image of the interface [15].

In acid-etching systems, it was possible to clearly identify the hybrid layer by such chemical or mechanical modification techniques. However, as for mild self-etching primer adhesive systems, SEM observation of the hybrid layer using such methods encountered limitations, since the hybrid layer classically observed as a distinguished layer in former generations of dentin adhesives was very thin for these systems. With the development of mild and simplified dentin bonding procedures, the observation method for interfaces between tooth substrates and adhesive resins has shifted from SEM to transmission electron microscopy (TEM), which provides an images with more details interfacial characteristics.

Koshiro et al. reported that the interface formed by the all-in-one adhesives were extremely thin (300 nm or less). They proposed that these adhesive systems should be categorized as "Nanointeraction Zone" type [16]. In this regard, a new approach for ultrastructural assessment of the interface was required.

4. SEM observation of the adhesive–dentin interface after acid–base challenge

Tsuchiya et al. reported the presence of an ABRZ below the hybrid layer with self-etching adhesive systems subjected to an artificial secondary caries attack [10]. However, morphological characteristics of this zone were highly material-dependent. The caries-like challenges at the adhesive–dentin interface can elucidate certain basic physico-chemical principles governing dissolution of the interfacial structures, which may be different from the *in situ* situation, due to lack of saliva and pH cycling.

Inoue et al. established the procedures for specimen preparation of the adhesive–dentin interface after acid–base challenge to visualize the secondary caries inhibition around the adhesive–dentin interface [11]. The sample preparation for SEM examination of the ABRZ, as they suggested, was illustrated in Fig. 1; human molars, obtained with the patients' informed consent, were sectioned at the mid coronal portion, vertical to the tooth axis, with a low-speed diamond saw (Isomet, Buehler, Lake Bluff, IL, USA) to obtain 1-mm thick dentin disks. The dentin surfaces were ground with #600-grit silicon carbide paper under running water. One surface of each disk was treated with an adhesive system according to the manufacturer's instructions. A flowable resin composite was then placed between pairs of the prepared dentin disks and

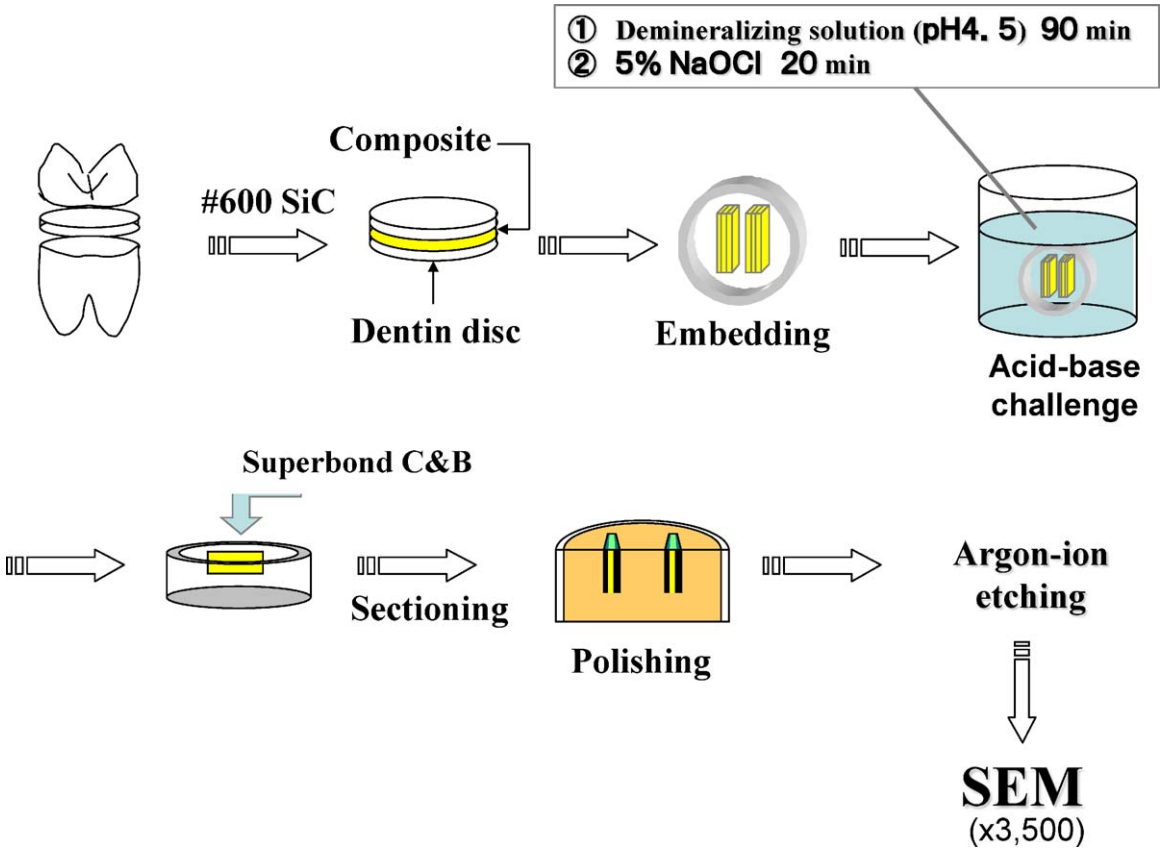

Figure 1 Specimen preparation for SEM observation (Inoue et al. [11]).

light-cured to make a dentin disk sandwich. After storing for 24 h in distilled water, each prepared specimen was sectioned perpendicular to the adhesive—dentin interface with a diamond saw and embedded with an epoxy resin (Epoxicure Resin, Buehler).

Each specimen was first stored in 100 ml of a buffered demineralizing solution, containing 2.2 mmol/L $CaCl_2$, 2.2 mmol/L NaH_2PO_4 and 50 mmol/L acetic acid adjusted at pH 4.5 for 90 min to create artificial secondary caries [17]. The specimens were then immersed in 5% NaOCl for 20 min in an attempt to remove any demineralized dentin collagen fibrils, and rinsed with running water for 30 s. Following this, a self-curing adhesive resin, 4-META/MMA-TBB resin (Super Bond C&B, Sun Medical, Moriyama, Japan), was applied without acid etching of the treated surface in order to prevent wear of the adhesive during polishing, as the edge of the adhesive could be torn away during specimen polishing [11]. After curing of the 4-META/MMA-TBB resin, the specimens were sectioned perpendicular to the dentin—adhesive interface, and reduced to approximately 1 mm thickness, then polished with diamond pastes (Struers A/S, Copenhagen, Denmark) down to 0.25 μm grit size. The polished surfaces were etched with an argon-ion beam (EIS-IE, Elionix, Tokyo, Japan) for 6 min to bring the hybrid layer into a sharp relief. Operating conditions for the argon-ion beam etching were an accelerating voltage of 1 kV and an ion current density of 0.2 mA/cm^2, with the ion beam directed perpendicular to the polished surface. The specimens were then gold-sputter coated, and morphological changes to the dentin—adhesive interface due to acid—base challenge were observed using a SEM (JSM-5310LV, JOEL, Tokyo, Japan). The procedure became a standard for ABRZ observation studies carried out since then. The adhesive materials used for morphologically analysis of an ABRZ are listed in Table 1.

5. Intact dentin vs caries-affected dentin

Inoue et al. observed the ultrastructures of the interface between intact or caries-affected dentin and a two-step self-etching primer adhesive system [11]. In that experiment, Clearfil SE Bond (Kuraray Medical, Tokyo, Japan) was used. This system is a fluoride-free two-step self-etching primer system. A good bonding to human sound dentin with a self-etching primer system has already been demonstrated in numerous laboratory studies [18—20] However, the tensile bond strength of a self-etching primer adhesive system to the caries-affected dentin was lower than that to the normal dentin [21,22].

Fig. 2 demonstrated SEM images at the interface between the adhesive and intact dentin (a, left) and between the adhesive and caries-affected dentin (b, right), respectively. In the results, the outer lesion, which is the dentin surface demineralized to simulate caries dentin lesion, was observed in both intact and caries-affected dentin. The depth of the outer lesion ranged from 10 μm to 15 μm, in which there was no difference between intact and caries-affected dentin. The adhesive demonstrated a good resistance to the acid—base challenge in the intact and caries-affected dentin specimens. The hybrid layer detected after argon-ion etching (H) was approximately 1 μm thick for the intact dentin, while a slightly thicker hybrid layer (H) was observed for the caries-affected dentin. These measurements were similar to the previous studies [22,23]. In addition, an ABRZ was observed beneath the hybrid layer (H) in SEM micrographs of both the intact and caries-affected dentin specimens. An ABRZ, approximately 1 μm thick, was observed beneath the hybrid layer (H) for the intact dentin, while a thicker ABRZ, approximately 1.5 μm thick, was created in the caries-affected dentin.

The mechanism of action for this two-step self-etching primer adhesive involves dissolution of the smear layer and demineralization of the underlying dentin by an acidic monomer, namely 10-methacryloxydecyl dihydrogenphosphate (MDP) in the primer, resulting in mild surface etching. Formation of an ABRZ by Clearfil SE Bond would be related to the penetration of the adhesive, but also to the quality of the hybrid layer (H).

Since the caries-affected intertubular dentin is already partially demineralized and more porous, caries-affected dentin is softer than normal dentin [24,25]. Thus, the intertubular dentin in caries-affected dentin should be more permeable to the primer than in normal dentin. Moreover, the smear layer of caries-affected dentin might be more porous than that of normal dentin [21]. Therefore, it was suggested that resin monomer could penetrate deeper into caries-affected dentin than intact dentin, resulting in a thicker hybrid layer (H) in caries-affected dentin.

In the mentioned study, the ABRZ and the surrounding lesion were also characterized by the nanoindentation technique. The mean values of the nanoindentation test (Fig. 3) demonstrated differences in the microhardness between the intact and caries-affected dentin specimens. As expected, microhardness of the intact dentin area was significantly higher than that of the caries-affected dentin [26]. Interestingly, the area 2 μm beneath the hybrid layer (H) in caries-affected dentin indicated a significantly higher microhardness compared to other dentin areas. This zone was coinciding with the ABRZ in the SEM observation (Fig. 2).

Dentin microhardness around the hybrid layer (H) was approximately 35 mgf/μm^2 for both the intact and caries-affected groups. In addition, microhardness values of the adhesive and the resin composite were approximately 27 mgf/μm^2 and 36 mgf/μm^2, respectively, in both intact and caries-affected dentin. Higher microhardness of the caries-affected dentin 2 μm beneath the hybrid layer supported the theory that the ABRZ was composed of penetrated monomer and dentin [11].

Secondary caries begins at the margin between dentin and the restorative material. In this study resin—dentin interface of the tested adhesive demonstrated resistance against acid—base challenge much more than both intact and caries-affected dentin. Thereby, the paper suggested that formation of an outer lesion away from the margins around the restoration should be primary caries rather than secondary caries [11]. In this regard, it could be stated that formation of an ABRZ is important in the prevention of secondary caries around a restoration.

6. Fluoride-releasing adhesive system

To date, manufacturers have been trying to develop various fluoride-releasing adhesive systems and resin composites

Table 1 Adhesive systems previously evaluated for ultrastructure of the acid–base resistant zone.

Adhesive system	Manufacturer	Components	Reference
Absolute 2	Dentsply-Sankin, Tokyo, Japan	acetone, water, pyrophosphatetetramethacrylate, 4-MET, PEM-F, UDMA, nanofillers, CQ, stabilizers	[42]
AQ Bond Plus	Sun Medical, Moriyama, Japan	Liquid: water, acetone, 4-META, polyfunctional acrylate, monomethacrylate, photo-initiators Sponge; p-toluene sulfinate salt, amine	[43]
Clearfil Protect Bond	Kuraray Medical Tokyo, Japan	Primer: MDPB, MDP, HEMA, hydropilic dimethacrylate, water Adhesive: MDP, HEMA, hydropilic dimethacrylate, PI, microfiller, NaF	[10,33,48]
Clearfil SE Bond	Kuraray Medical Tokyo, Japan	Primer: MDP, HEMA, hydropilic dimethacrylate, water, PI Adhesive: MDP, HEMA, hydrophilic dimethacrylate, PI, Bis-GMA, microfiller	[10,11,33,34,43,48]
Clearfil Tri-S Bond	Kuraray Medical, Tokyo, Japan	MDP, HEMA, Bis-GMA, hydrophobic dimethacrylate, sillanated colloidal silica, camphorquinone, ethanol, water	[40]
FL-Bond	Shofu Kyoto, Japan	FB Primer A: water, solvent, initiator FB Primer B: 4-AET, 4-AETA, HEMA, UDMA, TEGDMA, initiator Bond: HEMA, UDMA, TEGDMA, F-PRG filler, photo-initiator	[34]
FL-Bond II	Shofu, Kyoto, Japan	Primer: carboxylic acid monomer, 6-MHPA, Water, solvent, photo-initiator Bond: HEMA, UDMA, TEGDMA, S-PRG filler, photo-initiator	[34]
G-Bond	GC, Tokyo, Japan	4-MET, phosphate acid monomer, UDMA, silica, photo-initiator, acetone, water	[40]
Reactmer Bond	Shofu, Kyoto, Japan	A: distilled water FASG, F-PRG filler, CA B: 4-AET, 4-AETA, HEMA, UDMA, Pl	[10]
Single Bond	3M ESPE, St. Paul, MN, USA	Etchant: 35% phosphoric acid gel Adhesive: Bis-GMA, HEMA, polyalkenoic acid copolymer, ethanol, water, PI	[10,43,48]
Super Bond C&B	Sun Medical, Moriyama, Japan	Conditioner: 10% citric acid with 3% ferric chloride Powder: PMMA, Liquid: MMA, 4-META Catalyst: TBB	[44]
SI-R20603 (experimental)	Shofu, Kyoto, Japan	solvent, water, phosphonic acid, monomer, carboxylic acid monomer, polymeric monomer, photo-initiator, others	[42]
Tokuyama Bond Force	Tokuyama Dental, Tokyo, Japan	isopropyl alcohol, water, 3D SR monomer, Bis-GMA, TEGDMA, HEMA, glass fillers, photo-initiators	[42]

Abbreviations: 4-AET (4-AETA): 4-acryloxyethyltrimellitic acid (anhydride); Bis-GMA: bisphenol-A-glycidyl dimethacrylate; CA: catalyst; CQ: camphorquinone; 3D-SR: 3D self-reinforcing; FASG: fluoroalminosilicate glass; HEMA: 2-hydroxyethyl methacrylate; MDP: 10-methacryloxydecyl dihydrogenphosphate; MDPB: 12-methacryloyloxydodecylpyridinium bromide; 6-MHPA: 6-methacryloyloxyhexcyl phosphonoacetate; 4-MET (4-META): 4-methacryloxyethyl trimellitate (anhydride); MMA: methylmethacrylate; PMMA: poly methylmethacrylate; PI: photo-initiator; PRG: pre-reacted glass ionomer (F: full, S: surface); PEM-F: fluoromethacryloxy cyclophosphazen; TEGDMA: triethylene glycol dimethacrylate; TBB: tri-n-butyl borane; UDMA: urethane dimethacrylate.

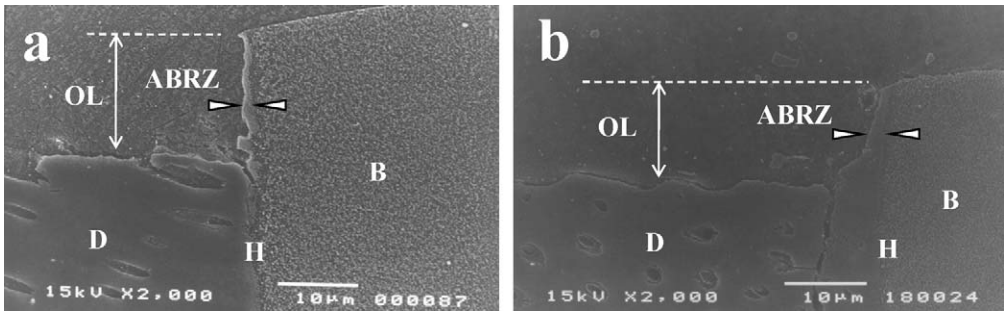

Figure 2 SEM observations at the interface between Clearfil SE Bond and dentin after acid—base challenge (3500×) (Inoue et al. [11]). (a) Intact dentin and (b) caries-affected dentin. OL: outer lesion; B: adhesive; H: hybrid layer; ABRZ: acid—base resistant zone; D: dentin. An acid—base resistant zone (ABRZ), approximately 1 μm thick, was observed beneath the hybrid layer (H) for the intact dentin (left), while a thicker acid—base resistant zone, approximately 1.5 μm thick, was created in the caries-affected dentin (right).

[27]. Studies have reported that fluoride-containing dentin adhesive may release fluoride into marginal gap and it may have a beneficial effect on the adjacent demineralized enamel and dentin [28,29]. Nakajima et al. [30] reported that the durability of dentin bonding created by a fluoride-releasing adhesive was improved for the six-month storage compared with a fluoride-free adhesive. They hypothesized that the fluoride somehow prevented the degradation of dentin, resulting in improvement of long-term stability at the adhesive interface. Toba et al. [31] stated using confocal laser-scanning microscopy that a fluoride-releasing adhesive system demonstrated the potential for artificial secondary caries inhibition around the restoration, although thickness of the inhibition zone had been relatively thinner than those created with the conventional glass-ionomer cements [8,32].

Knowing that the ABRZ was basically different in nature from the fluoride-inhibition zone, questions were raised on the possible effect of fluoride release from an adhesive on the formation of the ABRZ. Shinohara et al. performed an experiment using different self-etching primer systems and reported that a thicker ABRZ adjacent to the hybrid layer could be observed only when a fluoride-releasing adhesive was used (Fig. 4a) [33].

In their study, Clearfil Protect Bond (Kuraray Medical) was used as the fluoride-releasing adhesive system. The primer of this system has an antibacterial monomer (MDPB) and the adhesive has a fluoride-releasing component (treated sodium fluoride). The SEM analysis showed that the adhesive layer and hybrid layer were not damaged after acid—base challenge. A thin hybrid layer, approximately 0.5 μm thick could be observed. As pointed out, the interfaces of Clearfil Protect Bond group sharply demonstrated formation of a thick ABRZ (over 1.0 μm thick) adjacent to the hybrid layer. In addition, the ABRZ observed a slope increase in the thickness from the top towards the bottom of the outer lesion. Thereby, it was speculated that the formation of a thick ABRZ was related with fluoride release in the adhesive resin [33], because of the formation of a thicker ABRZ took place only when the fluoride-containing adhesive was used.

Iida et al. verified the dentin bond performance and the formation of the ABRZ at the bonded interface of two-step self-etching primer adhesive systems, Clearfil SE Bond, FL-Bond and FL-Bond II (both the latter by Shofu Inc., Kyoto, Japan) [34]. FL-Bond and FL-Bond II are fluoride-releasing systems, which have fluoride-releasing components of F-PRG filler and S-PRG filler, respectively. These filler particles were created by pre-reacted glass ionomer (PRG) technology [35,36]. Similar to the observation of the former study by Shinohara and others, the ABRZ of FL-Bond II sloped and increased in thickness from the top to the end of outer lesion (Fig. 4b), while the ABRZs of Clearfil SE Bond and FL-Bond were parallel to the hybrid layer and homogeneous. It was speculated that the gradual increase in the ABRZ thickness

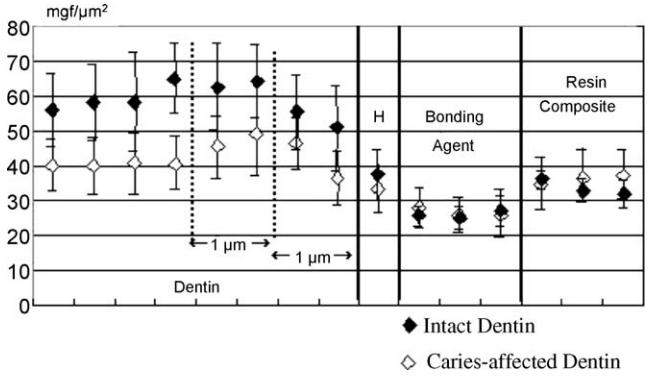

Figure 3 Mean value of microhardness at the adhesive—dentin interface (Inoue et al. [11]). Adhesive: Clearfil SE Bond. The area 2 μm beneath the hybrid layer (H) in caries-affected dentin indicated a significantly higher microhardness compared to other dentin areas. This zone was coinciding with the acid—base resistant zone (ABRZ) in the SEM observation (see Fig. 2).

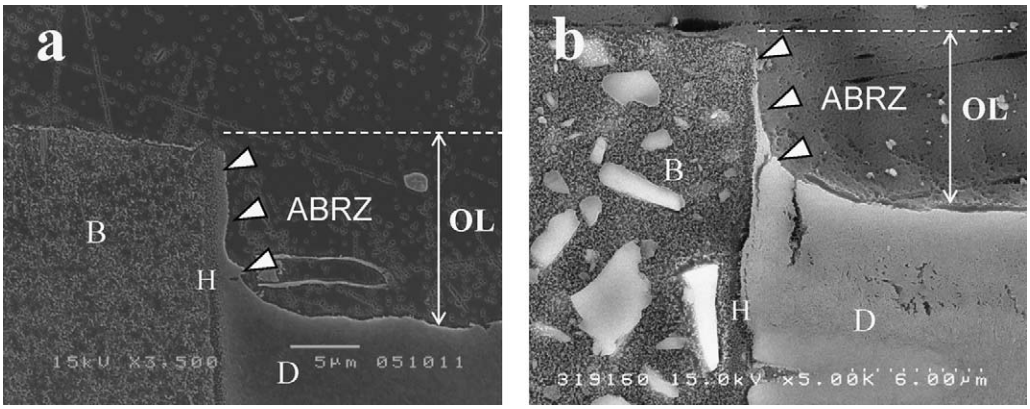

Figure 4 SEM observations at the interface between a fluoride-releasing adhesive and dentin after acid—base challenge. (a) Clearfil Protect Bond (3500×) (Shinohara et al. [33]) and (b) FL-Bond II (5000×) (Iida et al. [34]). OL: outer lesion; B: adhesive; H: hybrid layer; ABRZ: acid—base resistant zone; D: dentin. ABRZ adjacent to the hybrid layer could be observed only when the fluoride-releasing adhesive was used.

was not observed in FL-Bond, which may have been due to insufficient fluoride release from the FL-Bond adhesive.

Adding up the findings of the mentioned studies, it was suggested that the ABRZ formation is due to the monomer penetration, and fluoride release contributes to the process.

7. All-in-one adhesive system

In order to simplify the bonding procedures, all-in-one adhesive systems have been developed and commercialized. All-in-one adhesives contain acidic monomers, water, and solvents in order to create a bond between tooth substrate and resin composite by a single step. These systems may be advantageous for clinicians in saving time. However, the adhesive resin layer of the all-in-one adhesives is permeable and allows the formation of a water channel or water tree [37,38].

Two well-known examples for these systems are Clearfil Tri-S Bond (Kuraray Medical) and G-Bond (GC Corp., Tokyo, Japan). They both are fluoride-free all-in-one adhesive systems, which contain acidic monomers of MDP and 4-META, respectively. As mentioned in previous sections, acidic monomers play roles to condition and prime dentin simultaneously. However, the acidity of these adhesive systems did not reach that of the etchants in the acid-etching systems, such as phosphoric and citric acids [39]. Therefore, all-in-one adhesive systems demineralize dentin partially, leaving mineral crystals in the hybrid layer.

Representative SEM pictures of the dentin—adhesive interfaces in Clearfil Tri-S Bond and G-Bond after acid—base challenge are shown in Fig. 5 [40]. For Clearfil Tri-S Bond (Fig. 5a) and G-Bond (Fig. 5b), the thickness of both adhesives was less than 10 μm, respectively. A hybrid layer distinguished by argon-ion beam etching (H) was hardly observed at the interface. An ABRZ was observed beneath the hybrid layer, which was approximately 1 μm thick (white triangles) for both materials. However, the thickness of the ABRZ was adhesive material-dependent.

In all-in-one adhesives, hydrophobic and hydrophilic resin components are intermixed prior to polymerization. Phase separation can occur as the solvent — alcohol or acetone — is evaporated. The larger portion of adhesive solvents is removed by air drying after adhesive application, but residual water still persists due to lowering of the vapor pressure of water by HEMA. It is known that monomers can infiltrate deeper than the hybrid layer, and that water inhibits polymerization of the adhesives. So it is possible to speculate that

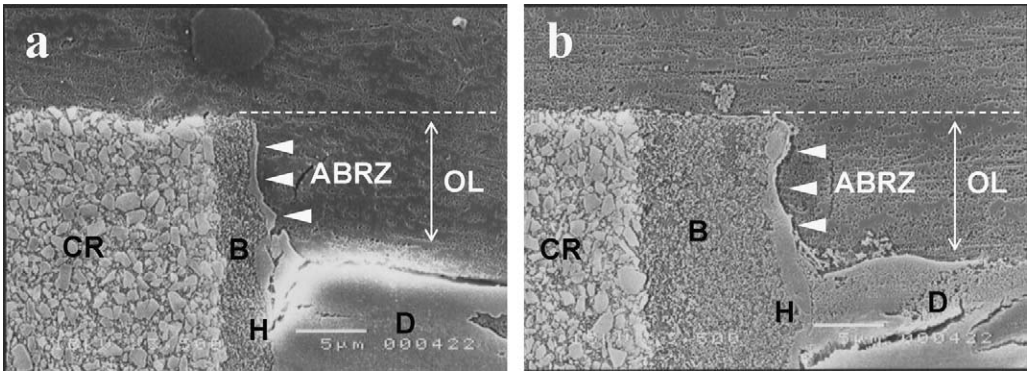

Figure 5 SEM observations at the interface between an all-in-one adhesive and dentin after acid—base challenge (3500×) (Nikaido et al. [40]) (3500×). (a) Clearfil Tri-S Bond and (b) G Bond. A hybrid layer distinguished by argon-ion beam etching (H) was hardly observed at the interface. An ABRZ was recognized beneath the hybrid layer, which was approximately 1 μm thick. It was very hard to detect the hybrid layer (H). An ABRZ (white triangles) was recognized beneath the hybrid layer.

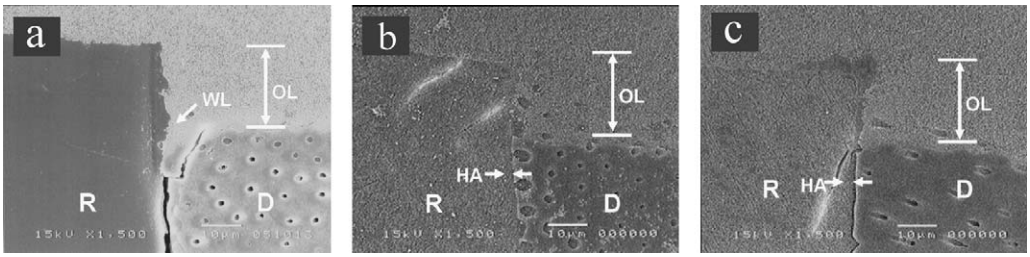

Figure 6 SEM observations at the interface between 4-META/MMA-TBB resin and dentin after acid—base challenge (3500×) (Takagaki et al. [44]). (a) No treatment (NT), (b) 65% phosphoric acid for 10 s (PA), and (c) 10% citric acid—3% ferric chloride (10-3) for 10 s. In the NT group, the hybrid layer was not created at the interface, however, wall lesion (WL) was observed along to the interface (a). Formation of the hybrid layer (H) was observed in both the PA (b) and 10-3 (c) groups; however, an acid—base resistant zone was not detected in any of the groups.

a weak area beneath the hybrid layer and ABRZ may be created, which is partially demineralized, while the penetrated monomers are not completely polymerized [41], due to the phase separation and water existence. In contrast to the ABRZ, it is reasonable to assume that this area is probably more vulnerable to acid challenge, resulting in the formation of typical erosion areas beneath the ABRZ in some adhesive systems [42,43].

8. Acid etching adhesive system

The ABRZ was discovered using a self-etching primer system. It was initially thought that the ABRZ may be specifically formed below the hybrid layer of adhesives that do not require acid etching of dentin. In order to probe this speculation and further clarify the attributes of this zone, Takagaki et al. evaluated the ultrastructural change of the adhesive—dentin interface after acid—base challenge using an acid etching adhesive system, 4-META/MMA-TBB resin with three different conditions [44]. Super Bond C&B is methylmethacrylate (MMA)-based, and contains a chemical initiator of a tri-n-butyl borane (TBB) derivative and a functional monomer of 4-methacryloxyethyl trimellitate anhydride (4-META), giving an excellent adhesion to dentin, when dentin surface is pretreated with citric acid solution containing ferric chloride [45—47].

The dentin surfaces received one of the following pretreatments: no treatment (NT), 65% phosphoric acid for 10 s (PA) or 10% citric acid—3% ferric chloride for 10 s (10-3). After application of PA or 10-3, the dentin surfaces were rinsed with water and gently air-dried. The mixture of liquid and powder of Superbond C&B was applied on dentin surface with a brush-on technique according to the manufacturer's instructions to bond a PMMA rod. The bonded specimens were left at room temperature for 30 min to secure the initial polymerization, and then stored in distilled water at 37 °C for 24 h.

The SEM photographs of the adhesive—dentin interface after acid—base challenge are revealed in Fig. 6. In the NT group, the hybrid layer was not created at the interface, however, wall lesion (WL) was observed along the interface. Formation of the hybrid layer was observed in both the 10-3 and PA groups; however, an ABRZ was not detected in any of the groups.

Nevertheless, without surface conditioning (NT), 4-META/MMA-TBB resin could not bond to dentin, because smear layer on the ground dentin surface prevented monomer penetration into underlying dentin. In the SEM observation after acid—base challenge, no hybrid layer formation was observed. However, wall lesions were found along the interface (Fig. 6a). Formation of wall lesion suggested that the interface without hybrid layer could not resist against acid—base challenge, indicating that a dentin margin without a hybrid layer would suffer secondary caries in the oral environment.

The hybrid layer was recognized in both 10-3 and PA. However, thickness of the hybrid layer with PA was 2 μm, while thickness with the 10-3 was 1 μm (Fig. 6b and c), the difference in thickness must be due to different acidity in two solutions.

Based on the results of the studies mentioned in the previous sections, an ABRZ was formed beneath the hybrid layer with a self-etching primer adhesive system. However, the ABRZ was not observed in the acid-etching system [10,43,44]. Schematic summary of the results of acid—base challenge was shown in Fig. 7 [44]. It was suggested that the existence of the ABRZ could be related to monomer penetration into the partially demineralized dentin, only when a self-etching primer adhesive system was used. Further evidence to support their speculations will be presented in the TEM observation section.

9. TEM observation

As pointed out in Section 1, TEM has become a valuable tool in the ultrastructural observation of resin—dentin interfaces. Waidyasekera et al. used two self-etch adhesive systems, and an acid-etch adhesive system in order to elucidate the laboratory artificial caries inhibition properties of the reinforced dentin with the evidence of TEM ultramorphology [48].

Sample preparation for TEM examination of the ABRZ in their experiment was basically similar to the procedure established for ABRZ observation using the SEM, as illustrated in Fig. 8. Dentin surfaces were treated with one of three dentin adhesives, Clearfil SE Bond, Clearfil Protect Bond, and Single Bond (3M ESPE, St. Paul, MN, USA). For the acid—base challenge, each specimen was stored in the buffered demineralizing solution for 90 min and 5% NaOCl for 20 min. After sectioning and drying, the TEM specimen preparation was performed in accordance with common procedures used for ultrastructural TEM examination of biological tissues. In this

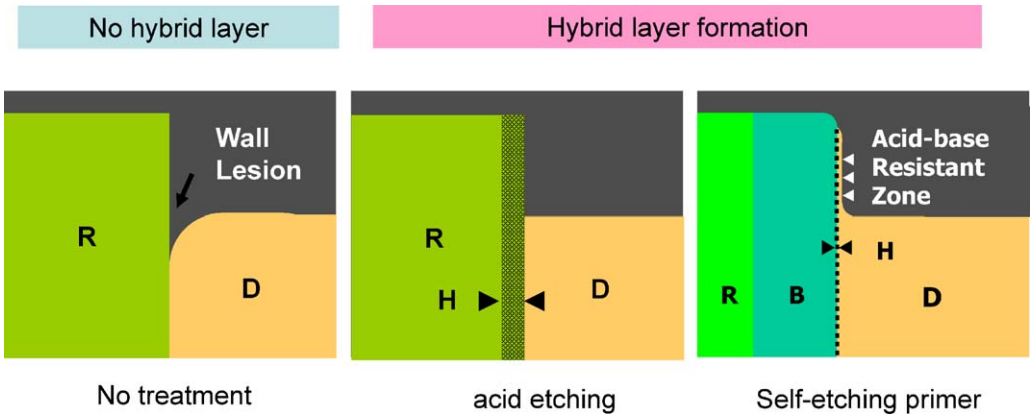

Figure 7 Schematic summary of the results of acid—base challenge (Takagaki et al. [44]). (a) No treatment, (b) acid-etching system, and (c) self-etching system. Hybridization is essential to prevent secondary caries. However, an acid—base resistant zone (ABRZ) was not observed in the acid-etching system. The ABRZ was formed only with a self-etching system.

regard, twenty specimens, each 100 nm in thickness, were observed under a transmission electron microscope (Hitachi H-600, Hitachi, Tokyo, Japan) in an accelerating potential of 75 kV and objective aperture diameter of 100 μm.

Results of the TEM observations of the adhesive—dentin interface after acid—base challenge are shown in Figs. 9 and 10. Moreover, selected area electron diffraction (SAED) patterns obtained from a small crystal cluster at the ABRZ in the two self-etching systems are shown in Fig. 11. The peak positions (*d*-spacing) 0 0 2 and 2 1 1 were identical, which suggested the presence of hydroxyapatite in the ABRZ.

The acid-etch system, Single Bond, did not show an ABRZ in this study (Fig. 9). This result was in accordance with the previous SEM studies on acid-etch systems [10,43]. As pointed in the previous section of the current review, when the surface is aggressively etched using an inorganic acid, the underlying dentin may become completely demineralized so deep that the bottom of the demineralized dentin would be inaccessible to complete impregnation by the resin [49]. In this case, a collagenous band at the base of the hybrid layer will not be impregnated by the resin. Signs of this incomplete resin penetration were observed as a nanoporous zone present at the base of the hybrid layer [50], which could become a pathway for nanoleakage fluid [51]. This incompletely sealed interface may have facilitated the acid penetration vigorously and given rise to the demineralization of the dentin below the hybrid layer.

On the other hand, this study evidently proved that the self-etch adhesive systems demineralize dentin mildly and partially, leaving hydroxyapatite crystals in the base of the hybrid layer (Fig. 10) [52]. Such residual apatite crystals may serve as a template for additional chemical reaction with the functional monomer, such as MDP in Clearfil SE Bond and Clearfil Protect Bond. It has been reported that MDP adhered

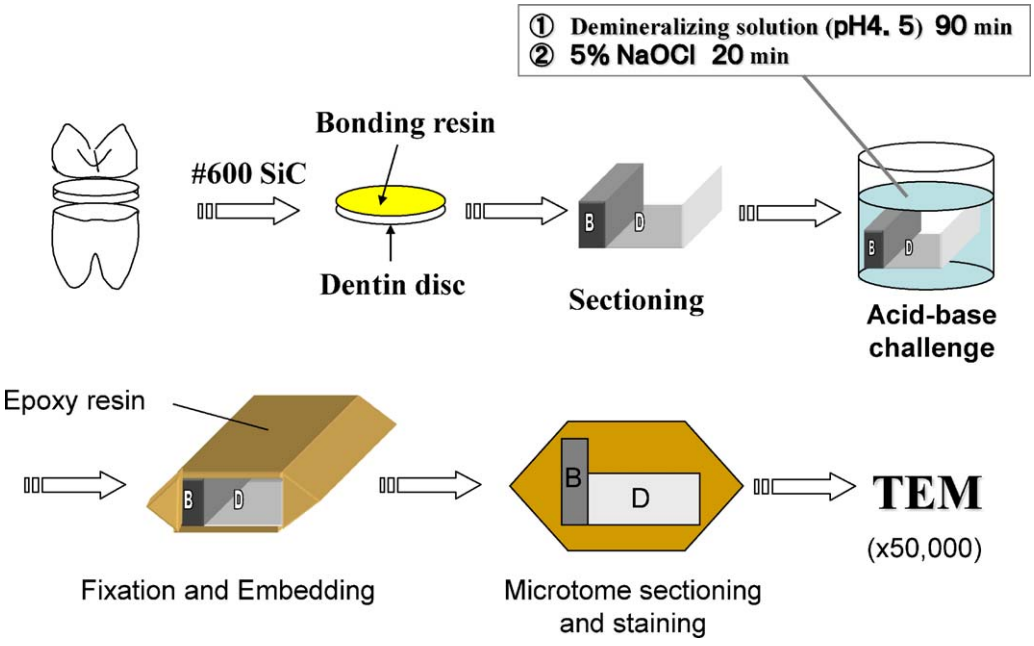

Figure 8 Specimen preparation for TEM observation (Waidyasekera et al. [48]).

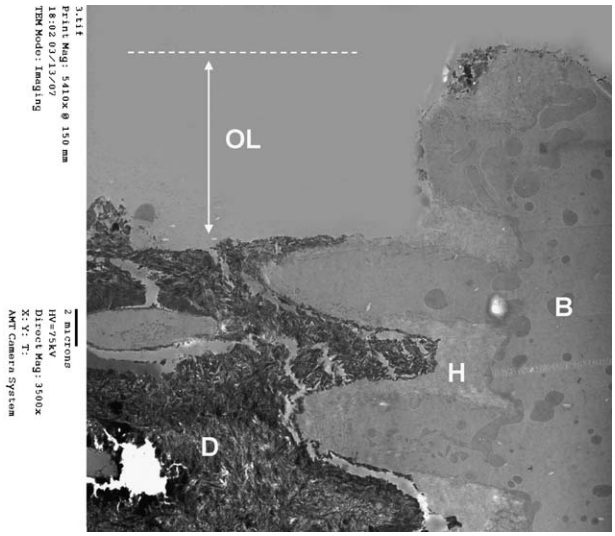

Figure 9 TEM observation at the interface between Single Bond and dentin after acid–base challenge (5000×) (Waidyasekera et al. [48]). OL: outer lesion; B: adhesive; H: hybrid layer; D: dentin. Single Bond did not show an acid–base resistant zone.

to hydroxyapatite readily and intensively [53], forming a less soluble salt, compared to the functional monomers, such as 4-MET (hydrated 4-META) and 2-(methacryloxy) ethylphenyl hydrogenphosphate (Phenyl-P) [52]. In these self-etch adhesives, the ABRZ was detected in the TEM observations, which were in accordance with the previous SEM studies

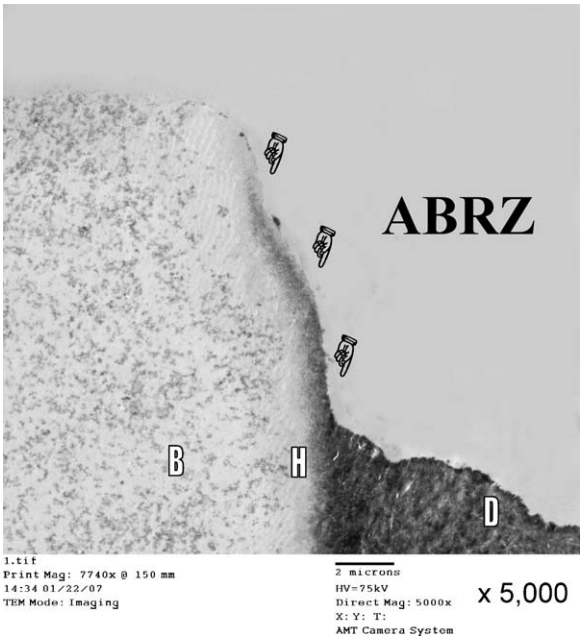

Figure 10 TEM observation at the interface between Clearfil SE Bond and dentin after acid–base challenge (5000×) (Waidyasekera et al. [48]). OL: outer lesion; B: adhesive; H: hybrid layer; ABRZ: acid–base resistant zone; D: dentin. In the top area of ABRZ, the electron dense region contained a few haphazardly arranged apatite crystallites with partial dissolution, while the bottom area of ABRZ showed densely packed crystallites. These regions with apatite crystallites were continuous with the dentin.

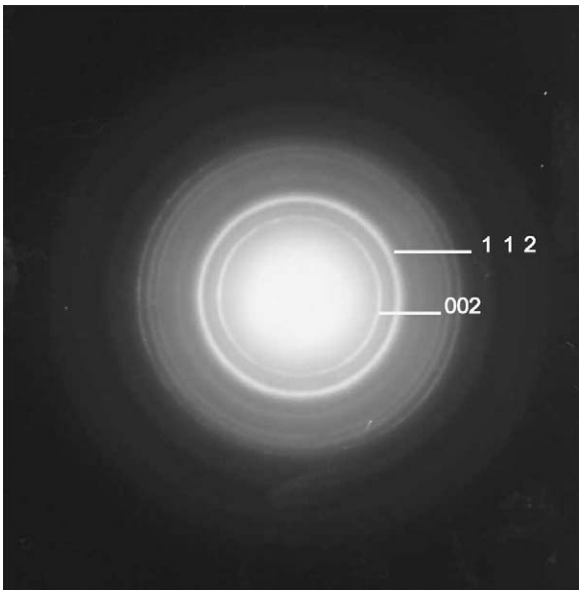

Figure 11 SAED pattern obtained for the apatite crystallites in the acid–base resistant zone of dentin treated with Clearfil SE Bond (Waidyasekera et al. [48]). The peak positions (*d*-spacing) 0 0 2 and 2 1 1 were identical, which suggested the presence of hydroxyapatite in the ABRZ.

[10,11,33,34,43]. The top area of the ABRZ was exposed to the acid attack for a longer period than the mid and bottom portions, where the electron dense region contained a few haphazardly arranged apatite crystallites with partial dissolution. On the other hand, the bottom area of ABRZ showed densely packed crystallites. These regions with apatite crystallites were continuous with the dentin, although the dentin below (outer lesion) is demineralized and dissolved.

As previously mentioned, Clearfil Protect Bond is a fluoride-ion releasing adhesive system [33]. Fluoride ions are reported to increase the rate of calcium phosphate crystallization and decrease the rate of apatite dissolution [54]. Dentin decalcified by acids is more sensitive to react with fluoride due to the increased porosity [55]. It was assumed in this study that the theory for reduced tendency of the apatite crystal dissolution in the presence of fluoride ions may be applicable for the formation of the thickest ABRZ observed with Clearfil Protect Bond, which has resulted to a better reinforced dentin. Formation of acid resistant fluoroapatite may be another possibility for this finding. But further differentiation among pure hydroxyapatites, carbonated apatites and fluoroapatites should be performed in the future.

This suggests that the ABRZ is not purely composed of dentin, but is rather a combination of dentin and the adjacent hybrid layer, which is said to be acid resistant [49] and created by penetration and polymerization of adhesive monomers after demineralization of dentin [53].

10. Concept of "Super Dentin" and clinical perspectives

As the studies reviewed suggested, the detailed mechanism for the ABRZ formation has not been understood yet; however, it is considered to be associated with monomer penetration deeper

than previously expected. In addition, formation of the ABRZ was strongly influenced by the fluoride ions released from a fluoride-containing adhesive system, which may accelerate remineralization reaction of dentin against acidic challenge, and create thicker ABRZ, compared with a fluoride-free adhesive system [33]. These findings suggest that the ABRZ is not solely composed of dentin, and a combination of dentin and the adjacent hybrid layer can better define this layer. These regions with apatite crystallites were expected to be continuous with the dentin. If the ABRZ is assumed made of resin-infiltrated dentin, the same chemical reaction of hydroxyapatite and an acidic monomer in the adhesive may take place in this zone, giving rise to the ability to resist against demineralization from an acid attack from the microorganisms in primary and secondary caries. Therefore, the reinforced dentin was proposed to be called as ''Super Dentin'' [40], which should be superior to the normal dentin mechanically, chemically and biologically. Using the self-etching technology, formation of ''Super Dentin'' may become a key strategy in preventive dentistry in the future.

Root surface coating with the dentin bonding systems is considered to be an effective measure for protection against caries, erosion and abrasion [56—58], as it provides a strong physical barrier with the formation of ''Super Dentin''. From the clinical stand point, to control the biofilm adherence on the coating material is also important to reduce caries risk in the oral environment [59]. A series of experimental coating materials with self-cleaning surface property have been developed, which demonstrated such surface property had good potential to inhibit biofilm adherence [60,61]. If such materials with a surface property could be combined with the current adhesive technology, the surface coating will become a promising therapy in preventive dentistry in the future.

Acknowledgements

This work was supported by the Global Center of Excellence (GCOE) Program; International Research Center for Molecular Science in Tooth and Bone Diseases at Tokyo Medical and Dental University, and a Grant-in Aid from the Japan Society for the Promotion of Science (JSPS No. 22592116).

References

[1] Nakabayashi N, Nakamura M, Yasuda N. Hybrid layer as a dentin bonding mechanism. J Aesthet Dent 1991;3:133—8.
[2] Pashley DH, Carvalho RM. Dentin permeability and dentine adhesion. J Dent 1997;25:355—72.
[3] Yuan Y, Shimada Y, Ichinose S, Sadr A, Tagami J. Effects of dentin characteristics on interfacial nanoleakage. J Dent Res 2007;86:1001—6.
[4] Mount GJ, Ngo H. Minimal intervention: a new concept for operative dentistry. Quintessence Int 2000;31:527—33.
[5] Mjör IA. Clinical diagnosis of recurrent caries. J Am Dent Assoc 2005;136:1426—33.
[6] Pereira PN, Inokoshi S, Tagami J. In vitro secondary caries in inhibition around fluoride releasing materials. J Dent 1998;26:505—10.
[7] Pereira PN, Inokoshi S, Yamada T, Tagami J. Microhardness of in vitro caries inhibition zone adjacent to conventional and resin-modified glass ionomer cements. Dent Mater 1998;14:179—86.
[8] Torii Y, Itota T, Okamoto M, Nakabo S, Nagamine M, Inoue K. Inhibition of artificial secondary caries in root by fluoride-releasing restorative materials. Oper Dent 2001;26:36—43.
[9] Okuda M, Pereira PN, Nikaido T, Tagami J. Evaluation of in vitro secondary caries using confocal laser scanning microscope and X-ray analytical microscope. Am J Dent 2003;16:191—6.
[10] Tsuchiya S, Nikaido T, Sonoda H, Foxton RM, Tagami J. Ultrastucture of the dentin—adhesive interface after acid base challenge. J Adhes Dent 2004;6:183—90.
[11] Inoue G, Tsuchiya S, Nikaido T, Foxton RM, Tagami J. Morphological and mechanical characterization of the acid—base resistant zone at the adhesive—dentin interface of intact and caries-affected dentin. Oper Dent 2006;31:466—72.
[12] Van Meerbeek B, Van Landuyt K, De Munck J, Hashimoto M, Peumans M, Lambrechts P, et al. Technique-sensitivity of contemporary adhesives. Dent Mater J 2005;24:1—13.
[13] Wang Y, Spencer P. Exploring the nature of acid-resistant hybrid layer with wet bonding. Oper Dent 2004;29:650—5.
[14] Gwinnett AJ, Kanca III J. Interfacial morphology of resin composite and shiny erosion lesions. Am J Dent 1992;5:315—7.
[15] Inokoshi S, Hosoda H, Harnirattisai C, Shimada Y. Interfacial structure between dentin and seven dentin bonding systems revealed using argon ion beam etching. Oper Dent 1993;18: 8—16.
[16] Koshiro K, Sidhu SK, Inoue S, Ikeda T, Sano H. New concept of resin—dentin interfacial adhesion: the Nanointeraction zone. J Biomed Mater Res B Appl Biomater 2006;77:401—8.
[17] Wefel JS, Heilman JR, Jordan TH. Comparisons of in vitro root caries models. Caries Res 1995;25:204—9.
[18] Inoue S, Vargas MA, Van Meerbeek B, Abe Y, Yoshida Y, Lambrechts P, et al. Micro-tensile bond strength of eleven modern adhesives to dentin. J Adhes Dent 2001;3:237—45.
[19] Yoshida Y, Nagakane K, Fukuda R, Nakayama Y, Okazaki M, Shintani H, et al. Comparative study on adhesive performance of functional monomers. J Dent Res 2004;83:454—8.
[20] Burrow MF, Kitasako Y, Thomas CD, Tagami J. Comparison of enamel and dentin microshear bond strengths of a two-step self-etching priming system with five all-in-one systems. Oper Dent 2008;33:456—60.
[21] Doi J, Itoya T, Torii Y, Nakabo S, Yoshiyama M. Micro-tensile bond strength of self-etching primer adhesive systems to human coronal carious dentin. J Oral Rehabil 2002;31:1023—8.
[22] Nakajima M, Ogata M, Okuda M, Tagami J, Sano H, Pashley DH. Bonding to caries-affected dentin using self-etching primers. Am J Dent 1999;12:309—14.
[23] Arrais CA, Giannini M. Morphology and thickness of the diffusion of resin through demineralized or unconditioned dentinal matrix. Pesqui Odontol Bras 2002;16:115—20.
[24] Ogawa K, Yamashita Y, Ichijo T, Fusayama T. The ultrastructure and hardness of the transparent layer of human carious dentin. J Dent Res 1983;62:7—10.
[25] Hamid A, Hume WR. Diffusion of resin monomers through carious dentin in vitro. Endod Dent Traumatol 1997;13:1—5.
[26] Nakajima M, Sano H, Burrow MF, Tagami J, Yoshiyama M, Ebisu S, et al. Tensile bond strength and SEM evaluation of caries-affected dentin using dentin adhesives. J Dent Res 1995;74: 1679—88.
[27] Imazato S, Torii M, Takatsuka T, Inoue K, Ebi N, Ebisu S. Bactericidal effect of dentin primer containing antibacterial monomer methacryloyloxydodecylpyridinium bromide (MDPB) against bacteria in human carious dentin. J Oral Rehabil 2001;28:314—9.
[28] Han L, Edward C, Okamoto A, Iwaku M. A comparative study of fluoride-releasing adhesive resin materials. Dent Mater J 2002;1:9—19.
[29] Ferracane JL, Mitchem JC, Adey JD. Fluoride penetration into the hybrid layer from a dentin adhesive. Am J Dent 1998;11: 23—8.

[30] Nakajima M, Okuda M, Ogata M, Pereira PNR, Tagami J, Pashley DH. The durability of a fluoride-releasing resin adhesive system to dentin. Oper Dent 2003;28:186—92.
[31] Toba S, Pereira PNR, Nikaido T, Tagami J. Effect of topical application of fluoride gel on artificial secondary caries inhibition. Int Chin J Dent 2003;3:53—61.
[32] Okuda M, Pereira PNR, Nikaido T, Tagami J. Evaluation of in vitro secondary caries using confocal laser scanning microscope and X-ray analytical microscope. Am J Dent 2003;16:191—6.
[33] Shinohara MS, Yamauti M, Inoue G, Nikaido T, Tagami J, Giannini M, et al. Evaluation of antibacterial and fluoride-releasing adhesive system on dentin—microtensile bond strength and acid—base challenge. Dent Mater J 2006;25:545—52.
[34] Iida Y, Nikaido T, Kitayama S, Takagaki T, Inoue G, Ikeda M, et al. Evaluation of dentin bonding performance and acid—base resistance of the interface of two-step self-etching adhesive systems. Dent Mater J 2009;28:493—500.
[35] Ikemura K, Tay FR, Kouro Y, Endo T, Yoshiyama M, Miyai K, et al. Optimizing filler content in an adhesive system containing prereacted glass-ionomer fillers. Dent Mater 2003;19:137—46.
[36] Kitayama S, Nikaido T, Ikeda M, Foxton RM, Tagami J. Enamel bonding of self-etch and phosphoric acid-etch orthodontic adhesive systems. Dent Mater J 2007;26:135—43.
[37] Tay FR, Pashley DH, Suh BI, Carvalho RM, Itthagarun A. Single-step adhesives are permeable membranes. J Dent 2002;30: 371—82.
[38] Yoshida E, Uno S. Voids formation along the bonding interface between a smeared dentin surface and all-in-one adhesives. Dent Mater J 2004;23:643—9.
[39] Hiraishi N, Kitasako Y, Nikaido T, Foxton RM, Tagami J, Nomura S. Detection of acidic diffusion through bovine dentine after adhesive application. Int Endod J 2004;37:455—62.
[40] Nikaido T, Weerasinghe DD, Waidyasekera K, Inoue G, Foxton RM, Tagami J. Assessment of the nanostructure of acid—base resistant zone by the application of all-in-one adhesive systems: super dentin formation. Biomed Mater Eng 2009;19: 163—71.
[41] Tay FR, King NM, Chan KM, Pashley DH. How can nanoleakage occur in self-etching adhesive systems that demineralize and infiltrate simultaneously? J Adhes Dent 2002;4:255—69.
[42] Nikaido T, Nishimura M, Iida Y, Inoue G, Tagami J. SEM observation of acid—base resistant zone in all-in-one adhesive systems. Adhes Dent 2007;25:197—203 (in Japanese with English abstract).
[43] Inoue G, Nikaido T, Foxton RM, Tagami J. The acid—base resistant zone in three dentin bonding systems. Dent Mater J 2009;28:717—21.
[44] Takagaki T, Nikaido T, Tsuchiya S, Ikeda M, Foxton RM, Tagami J. Effect of hybridization on bond strength and adhesive interface after acid—base challenge using 4-META/MMA-TBB resin. Dent Mater J 2009;28:185—93.
[45] Nakabayashi N, Takeyama M, Kojima K, Masuhara E. Studies on dental self-curing resins. Adhesion of 4-META/MMA-TBB resin to pretreated dentine. J Dent Mater 1982;1:74—7.
[46] Nakabayashi N, Kojima K, Masuhara E. The promotion of adhesion by the infiltration of monomers into tooth substrates. J Biomed Mater Res 1982;16:265—73.
[47] Kadoma Y, Imai Y. Effect of ferric salts on polymerization of MMA by TBBO in the presence of a collagen sheet—a model study the mechanism of adhesion of MMA resin to dentin. J Dent Mater 1988;7:817—23.
[48] Waidyasekera K, Nikaido T, Weerasinghe DS, Ichinose S, Tagami J. Reinforcement of dentin in self-etch adhesive technology: a new concept. J Dent 2009;37:604—9.
[49] Van Meerbeek B, Conn Jr LJ, Duke ES, Eick JD, Robinson SJ, Guerrero D. Correlative transmission electron microscopy examination of nondemineralized and demineralized resin—dentin interfaces formed by two dentin adhesive systems. J Dent Res 1996;75:879—88.
[50] Sano H, Shono T, Takatsu T, Hosoda H. Microporous dentin beneath resin-impregnated layer. Oper Dent 1994;19:59—64.
[51] Sano H, Takatsu T, Ciucchi B, Horner JA, Matthews WG, Pashley DH. Nanoleakage: leakage with in the hybrid layer. Oper Dent 1995;20:18—25.
[52] Fukegawa D, Hayakawa S, Yoshida Y, Suzuki K, Osaka A, Van Meerbeek B. Chemical interaction of phosphoric acid ester with hydroxyapatite. J Dent Res 2006;85:941—4.
[53] Yoshida Y, Nagakane K, Fukuda R, Nakayama Y, Okazaki M, Shintani H. comparative study on adhesive performance of functional monomers. J Dent Res 2004;83:454—8.
[54] White DJ, Nelson DGA, Faller RV. Mode of action of fluoride: application of new techniques and test methods to the examination of the mechanism of action of topical fluoride. Adv Dent Res 1994;8:166—74.
[55] Feuerstein O, Matalon S, Slutzky H, Weiss EI. Antibacterial properties of self-etching dental adhesive systems. J Am Dent Assoc 2007;138:349—54.
[56] Kaneshiro AV, Imazato S, Ebisu S, Tanaka S, Tanaka Y, Sano H. Effects of a self-etching resin coating system to prevent demineralization of root surfaces. Dent Mater 2008;24:1420—7.
[57] Tajima K, Nikaido T, Inoue G, Ikeda M, Tagami J. Effects of root dentin surface coating with adhesive materials to prevent demineralization of root surfaces. Dent Mater J 2009;28:578—86.
[58] Sundaram G, Wilson R, Watson TF, Bartlett DW. Effect of resin coating on dentine compared to repeated topical applications of fluoride mouthwash after an abrasion and erosion wear regime. J Dent 2007;35:814—8.
[59] Daneshmehr L, Matin K, Nikaido T, Tagami J. Effect of root dentin surface coating with all-in one adhesive materials on biofilm adherence. J Dent 2008;36:33—41.
[60] Gyo M, Nikaido T, Okada K, Yamauchi J, Tagami J, Matin K. Surface response of fluorine polymer-incorporated resin composites to cariogenic biofilm adherence. Appl Environ Microbiol 2008;74:1428—35.
[61] Okada A, Nikaido T, Ikeda M, Okada K, Yamauchi J, Foxton RM, et al. Inhibition of biofilm formation using newly developed coating materials with self-cleaning properties. Dent Mater J 2008;27:565—72.