DOI: 10.1111/j.1365-263X.2007.00909.x

Effect of acid etching time on bond strength of an etch-and-rinse adhesive to primary tooth dentine affected by amelogenesis imperfecta

NORIKO HIRAISHI, CYNTHIA KAR YUNG YIU & NIGEL MARTYN KING

Paediatric Dentistry and Orthodontics, Faculty of Dentistry, The University of Hong Kong, Prince Philip Dental Hospital, 34 Hospital Road, Hong Kong

International Journal of Paediatric Dentistry 2008; 18: 224–230

Background. Dentine affected by amelogenesis imperfecta (AI) is histologically altered following loss of the hypoplastic enamel and becomes hypermineralized, which would make bonding less predictable.
Aim. This study examined the effect of etching time on the microtensile bond strength (μTBS) to AI-affected primary dentine.
Design. Flat coronal dentine surface was obtained from extracted AI-affected and noncarious primary molars. Teeth were etched either for 15 or for 30 s using 34% phosphoric acid. Prime & Bond® NT™ (Dentsply De Trey), an etch-and-rinse adhesive, was applied to dentine surfaces, air-dried and light-cured, followed by composite build-ups. The bonded teeth were sectioned into beams of 0.8 mm^2 and stressed to failure under tension. Representative fractured beams from each group were examined under scanning electron microscopy.
Results. The extended etching time had an adverse effect on the μTBS for the normal dentine, while no significant difference was found for AI-affected dentine. When the AI-affected dentine was etched for 30 s, the fracture occurred in the demineralized dentine at the base of the hybrid layer.
Conclusion. Bonding to AI-affected dentine compromised the bonding of the etch-and-rinse adhesive. The bonding could not be improved by increasing etching time.

Introduction

Amelogenesis imperfecta (AI) affects the deposition, mineralization and maturation of enamel in both the primary and the permanent dentitions, as a result of specific genetic defects[1]. The diagnosis of AI frequently presents with sensitive and discoloured teeth. Poor dental aesthetics is the result of surface roughness, staining and abnormal crown shapes from enamel loss. Clinical management is considered to improve the poor appearance and function of the affected teeth using bonded restorations[2,3].

Histochemical study of dentine affected by hypocaclified AI demonstrated high calcium levels in response to the enamel disorder[4]. The morphological pattern of the affected dentine is similar to sclerotic dentine in terms of hypermineralization[4]. Dentine exposed to the oral environment undergoes compositional and morphological transformations, resulting in thickening of the peritubular dentine and partial obliteration of the dentine tubules[5]. These previous studies have shown a hypermineralized layer on the surface of etched sclerotic dentine, along with occlusion of dentinal tubules by mineral salts[5]. This layer was thought to be highly acid resistant, impairing or even preventing resin tag formation into the tubules. The high mineral content in sclerotic dentine compromises the formation of hybrid layer and results in a low bond strength[6,7]. Previously, it has been reported that the compromised bond strength to sclerotic dentine could be improved by extending the etching time[8,9]. It is questionable, however, whether the extended etching time would be effective to primary dentine that was affected by AI.

In general, the bond strength of the primary teeth was lower than that of the permanent teeth[10–13]. The primary teeth have different micromechanical[14] and histological[15] characteristics

Correspondence to:
Dr Noriko Hiraishi, Paediatric Dentistry and Orthodontics, Faculty of Dentistry, The University of Hong Kong, Prince Philip Dental Hospital, 34 Hospital Road, Hong Kong.
E-mail: hiraishinoriko@yahoo.com

from permanent teeth. The differences in the amount of mineral components, morphology and structure between primary and permanent are thought to be responsible for such low bond strengths. Greater tubular diameter and density have been reported for primary teeth, resulting in a reduced area of intertubular dentine for bonding[16]. The concentration of calcium and phosphate in peritubular and intertubular dentine is lower in primary teeth than that in permanent teeth[17]. The dentine of primary teeth is more reactive to acidic conditioners[18,19], due to the lower degree of mineralization[14]. A scanning electron microscopic study has demonstrated that hybrid layers formed in dentine of primary teeth were nearly 25–30% thicker than those of permanent dentine using identical acid etching times[18]. Consequently, shortening of the acid etching time has been recommended to prevent over-etching of dentine in primary teeth[19].

A few studies have reported high failure rates in resin–dentin bonding to AI-affected teeth[2,3,20]. Deproteinization using sodium hypochlorite (NaOCl) was reported as an effective treatment in enhancing the enamel bonding in hypocalcified AI enamel, while it showed adverse effect on AI dentine[20]. Chemical and morphological differences between sound and AI-altered teeth accounted for this failure[2,3]. Thus, bonding to morphologically altered tooth structure is challenging and complicated.

The aim of this study was to evaluate the microtensile bond strengths of an etch-and-rinse adhesive system to dentine from primary teeth with AI using different etching times (15 vs. 30 s). The null hypotheses to be tested were that the bond strength to exposed AI-affected dentine would not differ from that of normal primary dentine, and that the extended etching time would increase the bond strength on altered dentine.

Materials and methods

Specimen preparation

Six primary molars were collected from two boys, aged 9 and 10 years, with AI. Patients with AI were diagnosed by clinical examination[21]. Examination of these primary molars showed that most of the enamel had been chipped away or worn off, exposing large areas of dentine with yellowish-brown discolouration. Comparable six extracted noncarious teeth were obtained from apparently healthy 9- and 10-year-old children and served as control. The teeth used in this research were exfoliated and collected after the patients' informed consent was obtained under a protocol approved by the Ethics Committee, Faculty of Dentistry, The University of Hong Kong. The extracted teeth were stored in 0.5% chloramine T at 4 °C, and were used within 1 month following extraction.

For the preparation of normal teeth, a flat mid-coronal dentine surface was created perpendicular to the tooth's longitudinal axis using a slow-speed diamond saw (Isomet, Buehler Ltd, Lake Bluff, IL, USA) to remove root, occlusal enamel, and superficial dentine under water lubrication. The surface was ground with 180-grit silicon carbide paper under running water for 30 s to create a smear layer of clinically relevant thickness that is produced with a coarse-grained diamond bur[22]. The teeth with AI was prepared without instrumentation, thus, the bonded surface was devoid of smear layer formation.

Bonding procedure

The teeth with AI and the normal teeth were divided into two groups depending the etching time. Caulk® Tooth Conditioner Gel (Dentsply De Trey, York, PA,USA), 34% phosphoric acid, was applied for 15 or 30 s, followed by water rinsing for 15 s and blot-drying. A two-step etch-and-rinse adhesive, Prime & Bond® NT™ (Dentsply De Trey, York, PA, USA), was applied to the dentine surfaces, air-dried, and light-cured using a quartz-tungsten-halogen light-curing unit (Optilux 500, Demetron Research Corp., Danbury, CT, USA) operated at 600 mW/cm^2. Resin composite build-ups were performed on the bonded specimens using a light-cured microhybrid composite (Filtek Z250, 3M ESPE, St Paul, MN, USA) in five 1-mm increments that were light-activated separately.

Microtensile bond strength testing

After storage in distilled water at 37 °C for 24 h, the bonded teeth were sectioned occluso-gingivally into serial slabs, and further sectioned into 0.9 × 0.9 mm composite-dentine beams, according to the 'nontrimming' technique of the microtensile test reported by Shono *et al.*[23]. The bonded interfacial line was carefully observed on the beams from sample with AI using a stereomicroscope (Nikon SMZ10, Tokyo, Japan) at ×30 magnification to examine whether the interfacial line ran homogeneously perpendicular to the longitudinal axis of the beam. Since μTBS testing requires bonded surface to be homogeneously flat and stressed perpendicularly to the surface, the samples with apparently nonhomogeneous interfacial lines were discarded. The six to nine beams, randomly selected from one bonded normal teeth, were subjected to the bond strength testing. The exact dimension of each beam was measured using a pair of digital caliper (Model CD-6BS; Mitutoyo, Tokyo, Japan). Each beam was attached to the test apparatus with a cyanoacrylate adhesive (Zapit, Dental Ventures of America, Corona, CA, USA) and stressed to failure using a universal testing machine Model 4440 (Instron, Inc., Canton, MA, USA) at a crosshead speed of 1 mm per min. The data obtained were analysed using a statistical software package (SigmaStat version 2.03, SPSS, Chicago, IL, USA). A two-way ANOVA test has been performed to see if any interaction between etching time and dentine (with AI-affected dentine or normal dentine), followed by Tukey's multiple comparison test at significant level of 0.05.

Fracture pattern analysis and microscopic observation on fractured beams

The failure modes of all specimens were examined using the stereomicroscope at ×30 for normal dentine. For AI-affected dentine, which was not polished with SiC paper and exhibited no streaks, higher magnification up to ×100 to distinguish the failure patterns was used. The questionable fractured surface was further confirmed under scanning electron microscopy (SEM) examination. They were classified as cohesive failure within the resin composite and/or resin adhesive (1), adhesive failure at resin–dentine interface (2), cohesive failure within dentine (3), mixed failure with 1 and 2 (4), and mixed failure with 2 and 3 (5). Each type of failure mode was expressed as a percentage of the total number of specimens in that group. Representative fractured beams from each group with tensile bond strengths that were close to the mean bond strength of that group were examined with SEM. The fractured surface was air-dried, sputter-coated with gold/palladium 200 s at 30 mA using a sputter coater (BAL-TEC SCD 005, BAL-TEC Limited. Liechtenstein), and examined using a SEM (Cambridge Stereoscan 440, Cambridge, UK) operating at 12 kV.

Results

The means μTBS and standard deviations for each dentine type and etching time are listed in Table 1. No specimens failed prematurely during sectioning and microtensile bond strength testing. The results of two-way ANOVA showed that main factors, dentine type ($P < 0.001$) and acid etching time ($P < 0.001$), had significant effect on bond strength. The interaction between dentine type and etching time was statistically significant ($P = 0.002$), indicating that the effect of etching time on μTBS was different for normal dentine and AI-affected dentine. Extending the etching time to 30 s had an adverse effect on the μTBS for the normal dentine and resulted in significantly lower bond strength for 30 s (24.9 ± 5.1 MPa) when compared to 15 s (31.8 ± 4.8 MPa). For AI dentine samples, the mean μTBS has no significant difference between 15 and 30 s ($P = 0.881$).

The failure mode distribution (%) was shown in Table 2. Extending the etching time to 30 s

Table 1. Microtensile bond strengths of etch-and-rinse adhesive to dentine after different etching times.

	Microtensile bond strength (MPa ± SD) Normal dentine	(*n* = the number of sample) AI-altered dentine
15 s etching	31.8 ± 4.8^{a} ($n = 25$)	19.8 ± 6.6^{c} ($n = 24$)
30 s etching	24.9 ± 5.1^{b} ($n = 25$)	21.1 ± 7.7^{bc} ($n = 23$)

Groups identified by the same letter are not significantly different ($P > 0.05$) by Tukey test. AI, amelogenesis imperfecta.

Table 2. Failure mode distribution (%).

	Etching time	Failure mode 1	2	3	4	5
Normal teeth	15 s	40.0	0.0	8.0	48.0	4.0
	30 s	28.0	12.0	8.0	28.0	24.0
AI-altered teeth	15 s	4.2	33.3	0	41.7	20.8
	30 s	0.0	26.1	4.3	47.8	21.7

Failure modes: 1, cohesive failure within the resin composite or resin adhesive; 2, adhesive failure at resin–dentine interface; 3, cohesive failure within dentine; 4, mixed failure with 1 and 2; 5, mixed failure with 2 and 3. AI, amelogenesis imperfecta.

on normal dentine resulted in an increase in mixed failure pattern at the interface and within dentine when compared to that of 15 s etching time. With AI-affected dentine, the majority of the fracture patterns were mixed failure within the adhesive resin and at the bonded interface as well as adhesive failure at the bonded interface regardless to etching time. This is followed by mixed failure at the bonded interface and within dentine.

Figures 1–3 show SEM micrographs of the dentine sides of representative fractured beams from normal dentine that was etched for 30 s, AI-affected dentine etched for 15 s, and AI-affected dentine etched for 30 s, respectively. Fractured dentine in Fig. 1 was categorized into a mixed failure with cohesive failure within resin adhesive and adhesive failure at resin–dentine interface. Fractured dentine in Figs 2 and 3 were categorized into a mixed failure with cohesive failure within dentine and adhesive failure at resin–dentine interface. Dentinal tubules were slightly identified, indicating that the fracture occurred at resin–dentine interface.

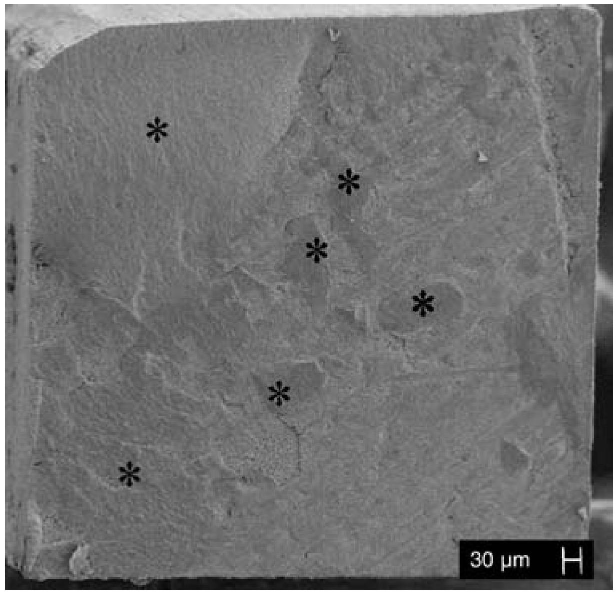

Fig. 1. SEM micrograph of the dentine side of a representative fractured beam from normal dentine that was acid-etched for 30 s. A mixed failure occurred within resin adhesive (asterisks) and at resin-dentine interface. Fracture at resin-detine interface was identified with streaks that were produced by polishing with a silicon carbide paper.

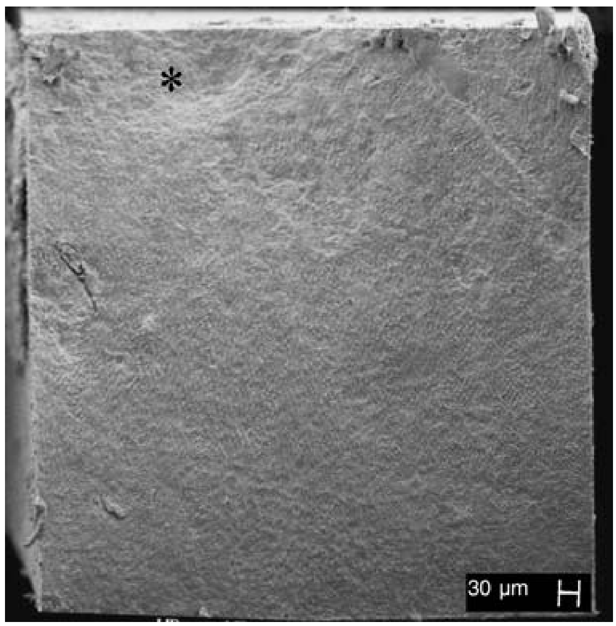

Fig. 2. SEM micrograph of the dentine side of a representative fractured beam from AI dentine that was acid-etched for 15 s. A mixed failure occurred within dentine (asterisk) and at resin-dentine interface. Note: since AI affected specimens were not prepared with a silicon carbide paper, no streaks were shown.

Discussion

Since the primary teeth used for this study were collected following exfoliation, the quality of dentine structure might be different from those which had been extracted for caries. As the size of primary teeth is considerably smaller than that of permanent teeth, the prepared coronal dentine surface might become very close to the pulpal chamber. The pulp chamber of exfoliating teeth, however, had been exposed to the environment, and pulpal fluid had not exuded on dentine surfaces. This implies that

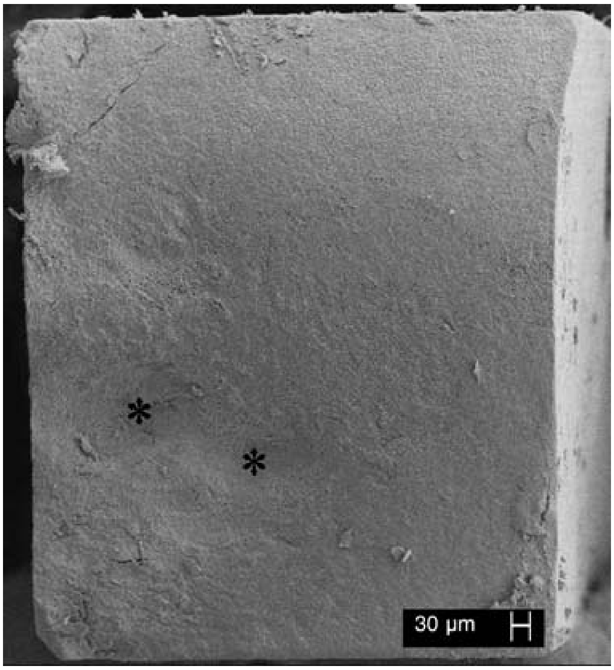

Fig. 3. SEM micrograph of the dentine side of a representative fractured beam from AI dentine that was acid-etched for 30 s. A mixed failure occurred within dentine (asterisks) and at resin-dentine interface.

the influence of fluid contamination from the pulp chamber on the bonding procedure would have been insignificant for the bond strengths in our study[10].

During tooth germ development, amelogenins, which are secreted on the predentine surface, can interact with odontoblasts or with products secreted from the processes on the dentine–enamel surface[24,25]. Thus, enamel proteins and dentine matrix proteins are involved in the process of dental development, causing the histological variation in dentine formation. The dentinal structure in AI teeth would be altered not only in developmental stage but also in the oral environment when the dentine surface is exposed following the loss of the overlying hypocalcified enamel.

In the previous study, scanning electron micrographs of dentine affected by hypocalcified AI revealed a thickening of the peritubular dentine, narrowing, and partial obliteration of the dentine tubules[4]. The borderline between intertubular and peritubular dentine could not be easily distinguished in many tubules[4]. A higher level of calcium was also reported for dentine with AI, when compared to normal dentine[4]. The structural changes and increased mineralization in dentine affected by hypocalcified AI are interpreted as mechanisms of response to the defective, friable enamel that is frequently lost[4]. These features should be comprehensively considered when planning to bond restorations to AI-affected dentine.

The concept of dentine adhesion is based on the formation of a hybrid layer; acid etching demineralizes superficial dentine and exposes collagen network, followed by resin monomers infiltration, facilitating micromechanical retention with the formation of resin tag[26]. TEM observation on cervical sclerotic dentine indicated insufficient resin tag and thin hybrid layer formation, which occurred as a result of inadequate intratubular and intertubular resin infiltration in acid-conditioned sclerotic dentine[27].

In our result, the bond strength of an etch-and-rinse adhesive to AI-affected dentine was significantly lower than that of normal dentine, thus the first hypothesis was rejected. The low μTBS, especially in AI-affected dentine that was etched for 15 s, may be directly attributed to the inadequate demineralization of the surface hypermineralized layer. This is consistent with previous studies on sclerotic dentine from permanent teeth[6,8,9]. Hypermineralized sclerotic tissue is probably more resistant to demineralization by acid etching[6]. It has been demonstrated that tubule obliteration in sclerotic dentine remained even after acid etching, thereby impairing or preventing resin infiltration into the tubules[6,28]. The resin–dentine interdiffusion zone is narrower in sclerotic substrate than that of normal dentine[29]. Increasing the acid etching time has been suggested as a way to improve bonding to sclerotic dentine[8,9]. On the other hand, it was reported that longer etching time produces excessive demineralization of normal dentine, hence hindering adequate resin impregnation[30]. The thick hybrid layers and incomplete resin infiltration into demineralized dentine compromised bond strength[30,31]. It must be noted that primary dentine demineralized more easily than permanent dentine when exposed to acid etching due to lesser mineralized structure than permanent dentine[18]. The etching time influences the depth of demineralization and the thickness of hybrid layer[32,33], although it was indicated that the thickness of the hybrid layer is not related to the bond strength[32,34].

Our study revealed that the μTBS of AI-affected dentine was only slightly increased by extending the etching time to 30 s; however, no significant difference was found between 15 and 30 s. Thus, the second hypothesis was accepted. We speculate that extending the acid etching time to 30 s produced more aggressive demineralization of the surface hypermineralized layer. Once the hypermineralized layer was dissolved by acid, over-etching of the underlying mineralized dentine might occur. The unsupported exposed collagen fibrils at the base of the hybrid layer might collapse following air drying of the dentine[35] and prevent resin infiltration due to a decreasing gradient of resin monomer diffusion within this matrix[36]. Hence, the non- or lesser-resin impregnated demineralized dentine at the base of the hybrid layer became the weakest zone within the bonded interface; consequently, bond failure was more likely to initiate from this zone[30]. The low bond strength observed in the over-etched AI-affected dentine may be due to the presence of this weak interface.

Based on the results of low μTBS for AI-affected dentine, it should be pointed out that distinct altered structure of AI-affected dentine may be responsible for the difficulty in achieving optimal adhesive bonding. Hypermineralization of the exposed dentine surface has been reported on teeth affected with AI[4]; however, the nature of this hypermineralized layer is unknown. These particular chemical, mechanical, and microstructural characteristics of AI-altered dentine make it difficult to produce adequate bond strength. Although extending the etching time may dissolve the surface hyperminelarized layer, it may eventually cause over-etching of the underlying mineralized dentine. If the dentin surface is over-etched, there is a risk that the resin monomers would not penetrate to the full depth of the demineralized dentin, causing a porous, nonresin infiltrated zone. In addition to etching time, there are other factors that may influence the formation of the hybrid layer and consequently, the bond strength, such as the type of acid agent[37,38] and the adhesive materials[10,39]. Within the limits of this study, we conclude that the bond strength of etch-and-rinse adhesive to AI-affected dentine could not be improved by extending the etching time. Further investigations are required to find other means to improve bonding to these morphologically altered tooth structures.

What this paper adds
- This study demonstrated that the microtensile strength of an etch-and-rinse adhesive to AI-affected dentine from primary teeth was lower than that to normal dentine.
- Increasing the etching time to 30 s could not improve the bond strength of an etch-and-rinse adhesive to AI-affected dentine.

Why this paper is important to paediatric dentists
- Paediatric dentists are often the first to encounter children with such anomalies. Bonded restorations are indicated clinically in a growing child to improve the appearance and function of teeth affected with AI.
- This paper showed that bonding to AI-affected primary tooth is more challenging than normal teeth. The reduced bond strength to AI-affected dentine from primary teeth could not be improved by extending the etching to 30 s as over-etching of the dentine matrix could compromise the bonding efficacy.

Acknowledgements

This study was supported in part, by grant 2003755/90800/08004/400/01, Faculty of Dentistry, The University of Hong Kong.

References

1 MacDougall M, Simmons D, Gu TT *et al*. Cloning, characterization and immunolocalization of human ameloblastin. *Eur J Oral Sci* 2000; **108**: 303–310.
2 Rada RE, Hasiakos PS. Current treatment modalities in the conservative restoration of amelogenesis imperfecta: a case report. *Quintessence Int* 1990; **21**: 937–942.
3 Seow WK. Clinical diagnosis and management strategies of amelogenesis imperfecta variants. *Pediatr Dent* 1993; **15**: 384–393.
4 Sanchez-Quevedo MC, Ceballos G, Garcia JM, Luna JD, Rodriguez IA, Campos A. Dentine structure and mineralization in hypocalcified amelogenesis imperfecta: a quantitative X-ray histochemical study. *Oral Dis* 2004; **10**: 94–98.
5 Van Meerbeek B, Braem M, Lambrechts P *et al*. Morphological characterization of the interface between resin and sclerotic dentine. *J Dent* 1994; **22**: 141–146.
6 Kwong SM, Tay FR, Yip HK, Kei LH, Pashley DH. An ultrastructural study of the application of dentine adhesives to acid-conditioned sclerotic dentin. *J Dent* 2000; **28**: 515–528.

7 Tay FR, Pashley DH. Resin bonding to cervical sclerotic dentin: a review. *J Dent* 2004; **32**: 173–196.
8 Lopes GC, Vieira LC, Monteiro S Jr, Caldeira de Andrada MA, Baratieri CM. Dentin bonding: effect of degree of mineralization and acid etching time. *Oper Dent* 2003; **28**: 429–439.
9 Lopes GC, Baratieri CM, Baratieri LN, Monteiro S Jr, Vieira LC. Bonding to cervical sclerotic dentin: effect of acid etching time. *J Adhes Dent* 2004; **6**: 19–23.
10 Burrow MF, Nopnakeepong U, Phrukkanon S. A comparison of microtensile bond strengths of several dentin bonding systems to primary and permanent dentin. *Dent Mater* 2002; **18**: 239–245.
11 Bordin-Aykroyd S, Sefton J, Davies EH. *In vitro* bond strengths of three current dentin adhesives to primary and permanent teeth. *Dent Mater* 1992; **8**: 74–78.
12 Salama FS, Tao L. Comparison of Gluma bond strength to primary vs. permanent dentin. *Pediatr Dent* 1991; **13**: 163–165.
13 Uekusa S, Yamaguchi K, Miyazaki M, Tsubota K, Kurokawa H, Hosoya Y. Bonding efficacy of single-step self-etch systems to sound primary and permanent tooth dentin. *Oper Dent* 2006; **31**: 569–576.
14 Angker I, Swain MV, Kilpatrick N. Micro-mechanical characterisation of the properties of primary tooth dentine. *J Dent* 2003; **31**: 261–267.
15 Shumikawa DA, Marshall GW, Gee L, Marshall SJ. Microstructure of primary tooth dentin. *Pediatr Dent* 1999; **21**: 39–44.
16 Marshall GW Jr, Marshall SJ, Kinney JH, Balooch M. The dentin substrate: structure and properties related to bonding. *J Dent* 1997; **25**: 441–458.
17 Hirayama A. Experimental analytical electron microscopic studies on the quantitative analysis of elemental concentrations in biological thin specimens and its application to dental science [in Japanese]. *Shikwa Gakuho* 1990; **90**: 1019–1036.
18 Nor JE, Feigal RJ, Dennison JB, Edwards CA. Dentin bonding: SEM comparison of the resin–dentin interface in primary and permanent teeth. *J Dent Res* 1996; **75**: 1396–1403.
19 Sardella TN, de Castro FL, Sanabe ME, Hebling J. Shortening of primary dentin etching time and its implication on bond strength. *J Dent* 2005; **33**: 355–362.
20 Saroglu I, Aras S, Oztas D. Effect of deproteinization on composite bond strength in hypocalcified amelogenesis imperfecta. *Oral Dis* 2006; **12**: 305–308.
21 Witkop CJ. Amelogenesis imperfecta, dentinogenesis imperfecta and dentin dysplasia revisited, problems in classification. *J Oral Pathol* 1989; **17**: 547–553.
22 Tani C, Finger WJ. Effect of smear layer thickness on bond strength mediated by three all-in-one self-etching priming adhesives. *J Adhes Dent* 2002; **4**: 283–289.
23 Shono Y, Ogawa T, Terashita M, Carvalho RM, Pashley EL, Pashley DH. Regional measurement of resin–dentin bonding as an array. *J Dent Res* 1999; **78**: 699–705.
24 Takano Y, Ozawa H, Crenshaw MA. Ca-ATPase and ALPase activities at the initial calcification sites of dentin and enamel in the rat incisor. *Cell Tissue Res* 1986; **243**: 91–99.
25 Paine CT, Paine ML, Luo W, *et al*. A tuftelin-interacting protein (TIP39) localizes to the apical secretory pole of mouse ameloblasts. *J Biol Chem* 2000; **275**: 22284–22292.
26 Nakabayashi N, Ashizawa M, Nakamura M. Identification of resin–dentin hybrid layer in vital human dentin created in vivo: durable bonding to vital human. *Quintessence Int* 1992; **23**: 135–141.
27 Kwong SM, Cheung GSP, Kei LH, *et al*. Micro-tensile bond strengths to sclerotic dentin using a self-etching and a total-etching technique. *Dent Mater* 2002; **18**: 359–369.
28 Marshall GW, Chang YJ, Saeki K, Gansky SA, Marshall SJ. Citric acid etching of cervical sclerotic dentin lesions: an AFM study. *J Biomed Mat Res* 2000; **49**: 338–344.
29 Prati C, Chersoni S, Mongiorgi R, Montanari G, Pashley DH. Thickness and morphology of resin-infiltrated dentin layer in young, old, and sclerotic dentin. *Oper Dent* 1999; **24**: 66–72.
30 Hashimoto M, Ohno H, Kaga M, *et al*. Over-etching effects on micro-tensile bond strength and failure patterns for two dentin bonding systems. *J Dent* 2002; **30**: 99–105.
31 Pioch T, Stotz S, Buff E, Duschner H, Staehle HJ. Influence of different etching times on hybrid layer formation and tensile bond strength. *Am J Dent* 1998; **11**: 202–206.
32 Bolanos-Carmona V, Gonzalez-Lopez S, Briones-Lujan T, De Haro-Munoz C, de la Macorra JC. Effects of etching time of primary dentin on interface morphology and microtensile bond strength. *Dent Mater* 2006; **22**: 1121–1129.
33 Malferrari S, Finger WJ, Garc'ýa-Godoy F. Resin bonding efficacy of Gluma 2000 to dentine of primary teeth: an in vitro study. *Int J Paediatr Dent* 1995; **5**: 73–79.
34 Hashimoto M, Ohno H, Endo K, Kaga M, Sano H, Oguchi H. The effect of hybrid layer thickness on bond strength; demineralized zone of hybrid layer. *Dent Mater* 2000; **16**: 406–411.
35 Pashley DH, Ciucchi B, Sano H, Horner JA. Permeability of dentin adhesive agents. *Quintessence Inter* 1993; **24**: 618–631.
36 Wang Y, Spencer P. Hybridization efficiency of the adhesive/dentin interface with wet bonding. *J Dent Res* 2003; **82**: 141–145.
37 Perdigao J, May KN, Wilder AD, Lopes M. The effect of depth of dentin demineralization on bond strengths and morphology of the hybrid layer. *Oper Dent* 2000; **25**: 186–194.
38 Perdigao J, Lambrechts P, Van Meerbeek B, Tome AR, Vanherle G, Lopes ALB. A morphological field emission SEM study of the effect of six phosphoric acid etching agents on human dentin. *Dent Mater* 1996; **12**: 262–271.
39 da Silva Telles PD, Aparecida M, Machado M, Nor JE. SEM study of a self-etching primer adhesive system used for dentin bonding in primary and permanent teeth. *Pediatr Dent* 2001; **23**: 315–320.