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Carious affected dentine: its behaviour in adhesive bonding

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ABSTRACT

Background: Carious affected dentine (CAD) represents a very common substrate in adhesive dentistry. Despite its ability to interact with adhesive systems, the intrinsic character of CAD leads to lower bonding compared with sound dentine, regardless of the adhesive systems used. This low bonding may be more susceptible to leakage and hydrolysis of the interface by matrix metalloproteinases (MMPs). This systematic review aimed to determine current knowledge of CAD bonding, together with bond strength and MMP inhibitors' ability to prevent hybrid layer instability.
Methods: MEDLINE/Pubmed, Scopus and The Cochrane Library databases were electronically searched for articles published from 1 January 1960 to 31 August 2014. Two reviewers independently screened and included papers according to predefined selection criteria.
Results: The electronic searches identified 320 studies. After title, abstract and full-text examinations, 139 articles met the inclusion criteria. Data highlighted that a poor resin saturation of the already demineralized collagen matrix in CAD is strictly related to nanoleakage in interdiffusion and is the basis of the progressive decrease in strength with hydrolysis by MMPs. The use of mild self-etching systems seems to be the more accredited method to establish bonding in CAD. Inhibitors of MMPs may ensure better performance of CAD bonding, allowing undisturbed remineralization of the affected matrix.
Conclusions: CAD bonding needs further understanding and improvement, particularly to enhance the strength and durability of the hybrid layer.

Keywords: Adhesion, carious affected hybrid layer, etch-and-rinse adhesives, matrix metalloproteinases, self-etching adhesives.

Abbreviations and acronyms: CAD = carious affected dentine; CID = caries infected dentine; ERA = etch-and-rinse adhesives; FTIR = Fourier-transform infrared imaging; HAP = hydroxyapatite; MMPs = matrix metalloproteinases; SEA = self-etching adhesives.

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INTRODUCTION

Caries is considered the most common process that changes the dentine substrate and results in the need for restoration in dentistry. It is caused by the biofilm, or dental plaque, that is the pathological stimulus to the bacterial attack of teeth.[1,2]

Carious dentine consists of a superficial first layer and a deeper second layer (Fig. 1).[3,4]

In the outer layer, or carious infected dentine (CID), the dentine becomes decomposed due to activation of matrix metalloproteinase (MMPs).[5,6] CID is also called the zone of destruction in the carious process because it loses the features of dentine completely. Collagen fibres degenerate with the disappearance of the cross-linkers of type I collagen, indicating irreversible denaturation of the matrix. Bacteria are frequently observed inside the tubules (Fig. 2).

As a consequence of the very low bonding capacity, CID is actually removed from the bottom of the excavated lesion.

Conversely, the deeper carious layer, or caries-affected dentine (CAD) is a remineralizable tissue.[4,7]

In CAD, the collagen matrix shows apatite crystals fitting to the fibrils, even if the secondary structure of collagen appeared slightly altered when compared to that of unaltered dentine (Fig. 3). Using Fourier-transform infrared imaging (FTIR), a loss of crystallinity in the mineral phase was observed, and also reduced mineral content and spectral changes in the secondary structure of the collagen.[8]

A common observation is the presence of tubular occlusions by the formation of mineral intratubular deposits of *Beta*-tricalcium phosphate, or *whitlockite* deposits.[9] Occlusions change the refractive index of the *lumen*, becoming similar to that of intertubular

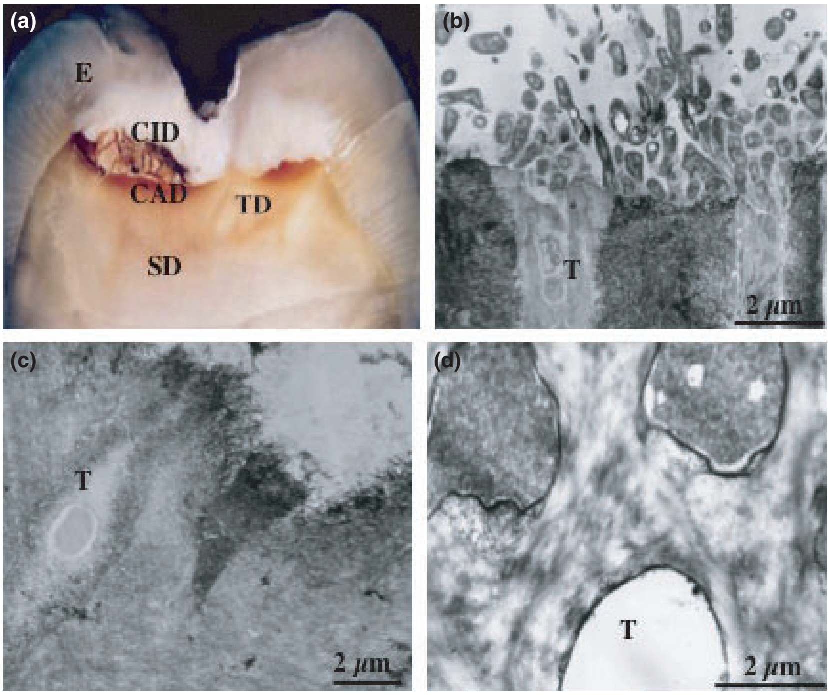

Fig. 1 Panel a is a light microscopy image of a carious process. Demineralization produced by carious bacteria has affected the enamel (E) and the upper part of the crown dentine (D). The outer layer of carious infected dentine (CID) appears degraded. Below this, the transparent layer (TD) of carious affected dentine (CAD) can be easily identified due to the higher chrome compared with the surrounding layers of CAD and unaltered dentine (UD). Panel b is a transmission electron microscopy (TEM) image of CID showing tubules (T) in a longitudinal section that have been invaded by bacteria. The intertubular dentine is degenerated and no collagen fibres can be discerned. In Panel c, CAD tubular *lumenes* (T) appear completely occupied by minerals. Tubules (T) in a transversal section in UD contain odontoblast and dentinal fluids.

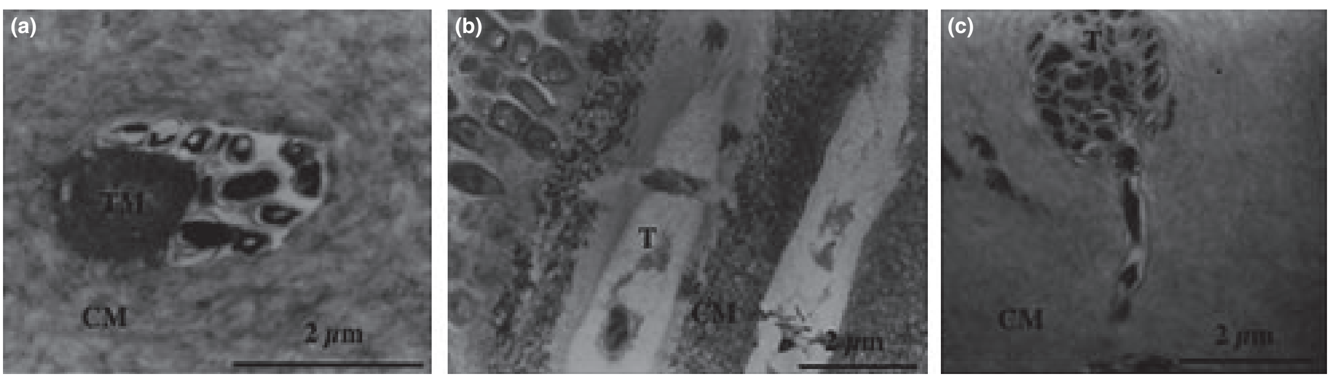

Fig. 2 CID: different aspects of bacterial progression. In Panel a, a slow bacterial progression leads to retraction of the odontoblast process and the simultaneous deposition of minerals (TM) within the dentinal tubules. Collagen fibrils can still be identified in the intertubular matrix. In Panel b the rapid progression of bacteria destroys the odontoblast process without tubular mineralization, leaving empty tubules called dead tracts. Intertubular collagen characteristics appear to have completely vanished. In Panel c, the dead tracts have been invaded by bacteria.

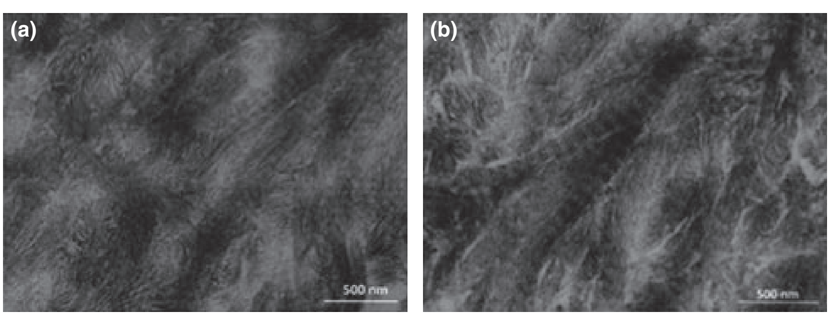

Fig. 3 Demineralized TEM sections of dentinal collagen. In Panel a, CAD fibres show loose banding and dense but not homogenous crystalline structures in comparison to the sound fibres. In Panel b, unaltered collagen fibres have dense transversal banding and needle-shaped densely packed apatite crystals with less mineral dense regions between them.

dentine. For this reason, the layer of tubular occlusion in CAD was named the *transparent layer*.[4]

Even if occlusions make the tubular *lumen* impermeable to dentinal fluids,[10] wetness is increased in intertubular affected dentine as a consequence of the fact that water replaces the minerals lost in equal volume. It leads to an increase of water content in CAD that varies from 14% to 53% compared with the 10% of sound dentine.[11] Wetness may make the saturation of hydrophobic resins more difficult, leading to evidence of porosities in CAD interdiffusion,[10] interpreted as water retention within the hybrid layer.

Moreover, the tubular occlusions hamper the percolation of resin monomers with tubular tag formation. Subsequently, a tight bond in CAD could be affected by the presence of water within the hybrid layer and the absence of micromechanical tag reinforcements.

Furthermore, the loss of minerals in the intertubular matrix has a negative effect on tensile strength and Knoop hardness becoming lower than that of unaltered dentine.[12–15] The reduction of these mechanical properties significantly influences a decrease of the mean elastic modulus and nanohardness in CAD when compared to unaltered tissue.[16,17]

As a result a global decrease in bond strength and durability of CAD interface has been reported in the literature, regardless of adhesive systems and bonding procedure.[18–20] Interferences in infiltration and reinforcement of the affected fibres due to the cavity smear layer, as well as the behaviour of the adhesive systems in CAD could also explain these findings.

All the above factors may favour instability and hydrolysis in the CAD hybrid layer by nanoleakage and degradation by MMPs.[21]

Therefore, despite the important developments in adhesion of the last decades, bonding in CAD requires further understanding and improvement. Several aspects of enhancing strength and durability of CAD bonding have to be clarified, including an understanding of the features of the surfaces exposed after cavity preparation and the influence of the characteristics of the adhesives. This increased understanding would be helpful in obtaining a tight resin/dentine interdiffusion, which is compatible with a stable bond as opposed to nanoleakage and hydrolysis by the host derived MMP.

The aim of this systematic review was to determine current knowledge of CAD bonding, together with bond strength and MMP inhibitors' ability to prevent hybrid layer instability.

MATERIALS AND METHODS

Search strategy

This systematic review was performed according to the PRISMA Statement.[22] A first systematic literature search for articles related to the bond strength and bond strength durability of CAD bonding, the effect of MMPs on bond stability and the effect of smear layer on bond strength to dentine, published between 1 January 1960 and 31 August 2014, was conducted in the databases of MEDLINE/PubMed, Scopus and The Cochrane Library, using combinations of the MeSH terms: [Caries-affected dentine] AND [Dental Adhesives Systems] OR [Dentine bonding] OR [Bond Strength] OR [Microtensile bond test] AND [Durability] OR [Nanoleakage] AND [Matrix Metalloproteinases] OR [Enzymatic degradation] AND [Smear Layer]. The search results were imported into a computerized database Review Manager 5.2. The search results from each of the electronic databases of MEDLINE/PubMed, Scopus and The Cochrane Library were combined, and duplicated publications were eliminated.

Inclusion and exclusion criteria for study selection

After completing the search, articles for review were selected based on: (1) original data protocols; (2) etch-and-rinse and self-etch adhesive systems; (3) studies on human permanent teeth; and (4) English language.

Studies were excluded if they were: (1) without original and/or actual data; (2) with data from previous publications; (3) opinion papers; and (4) Editorials.

By removing irrelevant citations according to the selected criteria, a preliminary set of potentially relevant publications was created.

Screening and selection

Using a screening guide based on eligibility criteria, two reviewers (RP and MM) independently screened the registered titles and abstracts, authors and references in two separate files (one including abstracts and the other excluding abstracts). The full text of all potentially eligible studies in at least one screening was retrieved. Reviewers then evaluated the full text for inclusion using a screening guide and a second reviewer (RP) screened all the findings. When disagreement occurred, a third reviewer (IM) was consulted.

Data extraction

An *ad hoc* data extraction form was designed to record data from the selected studies. For the smear layer effect on bond strength to dentine articles were recorded: authors, years, adhesive material, classification, manufacturer, bond strength test method, specimen staging method, bond strength reduction expressed in MPa (Table 1).[20,23–43] For bond strength

Table 1. Systematic review of smear layer effect on bond strength to dentine papers

Author	Ref.	Year	Adhesive material	Classifications	Manufacturer	Test method (MPa)	Specimen staging method (dentine surface preparation)	Bond strength reduction (means ± SD)
								Pertac II – Hytac ApliTip
Frankenberger *et al.*	23	2001	Adper Prompt L-Pop	1-Step Self-Etch	3M ESPE	µTBS	#600 Silicon Carbide Paper	5.2 (± 6.1) – 13.5 (± 6.3)
			Prime & Bond NT	2-Step Etch-and-Rinse	Dentsply			19.0 (± 3.9) – 19.4 (± 7.6)
Koibuchi *et al.*	24	2001	Clearfil Liner Bond 2	2-Step Self-Etch	Kuraray	µTBS	#180 Silicon Carbide Paper	10.0 (± 7.2)
							#600 Silicon Carbide Paper	28.5 (± 5.2)
Ogata *et al.*	25	2001	Clearfil Liner Bond 2	2-Step Self-Etch	Kuraray	µTBS	#600 Silicon Carbide Paper	40.4 (± 9.7)
			Clearfil Liner Bond 2V	2-Step Self-Etch	Kuraray			54.4 (± 11.3)
			Clearfil SE Bond	2-Step Self-Etch	Kuraray			47.0 1 (± 3.7)
Chaves *et al.*	26	2002	Prime & Bond NT	2-Step Etch-and-Rinse	Dentsply	µTBS	#600 Silicon Carbide Paper	40.2 (± 6.1)
			Clearfil Mega Bond	2-Step Self-Etch	Kuraray			21.1 (± 4.3)
			Etch & Prime 3.0	1-Step Self-Etch	Degussa AG			12.8 (± 4.2)
Tani *et al.*	27	2002	AC Bond	1-Step Self-Etch	Heraeus	µSBS	#180 Silicon Carbide Paper	17.3 (± 2.0)
							#600 Silicon Carbide Paper	18.6 (± 1.9)
			AQ Bond	1-Step Self-Etch	Sun Medical		#180 Silicon Carbide Paper	15.7 (± 2.7)
							#600 Silicon Carbide Paper	18.7 (± 1.6)
			Adper Prompt L-Pop	1-Step Self-Etch	3M ESPE		#180 Silicon Carbide Paper	17.3 (± 1.3)
							#600 Silicon Carbide Paper	18.2 (± 1.5)
Chan *et al.*	28	2003	ABF System	2-Step Self-Etch	Kuraray	µTBS	#180 Silicon Carbide Paper	37.1 (± 9.5)
			Imperva Fluoro Bond	2-Step Self-Etch	Shofu			26.6 (± 7.6)
			One-Up Bond F	1-Step Self-Etch	Tokuyama			32.2 (± 7.9)
			AQ Bond	1-Step Self-Etch	Sun Medical			18.0 (± 3.8)
Oliveira *et al.*	29	2003	Adper Single Bond	2-Step Etch-and-Rinse	3M ESPE	µSBS	#600 Silicon Carbide Paper	25.4 (± 6.8)
			Clearfil SE Bond	2-Step Self-Etch	Kuraray			42.0 (± 7.5)
Dias *et al.*	30	2004	Adper Single Bond	2-Step Etch-and-Rinse	3M ESPE	µTBS	#600 Silicon Carbide Paper	31.7 (± 7.5)
			Clearfil SE Bond	2-Step Self-Etch	Kuraray			59.3 (± 12.4)
			ABF System	2-Step Self-Etch	Kuraray			45.6 (± 7.5)
			Imperva Fluoro Bond	2-Step Self-Etch	Shofu			33.8 (± 12.3)
			One-Up Bond F	1-Step Self-Etch	Tokuyana			33.4 (± 6.1)
Toledano *et al.*	31	2004	Clearfil SE Bond	2-Step Self-Etch	Kuraray	µTBS	#180 Silicon Carbide Paper	44.0 (± 12.1) (Blot-dried)
								55.9 (± 9.9) (Air-dried)
Kenshima *et al.*	32	2005	Clearfil SE Bond	2-Step Self-Etch	Kuraray	µSBS	#600 Silicon Carbide Paper	40.7 (± 5.5)
			Optibond Solo Self-Etch- Primer	2-Step Self-Etch	Kerr			35.7 (± 3.9)
			Tyrian SPE and One Step Plus–TY	2-Step Self-Etch	Bisco			22.9 (± 2.8)
			Adper Single Bond	2-Step Etch-and-Rinse	3M ESPE			40.8 (± 7.3)
			Scotchbond Multipurpose	2-Step Etch-and-Rinse	3M ESPE			41.0 (± 3.2)
Reis *et al.*	33	2005	Clearfil SE Bond	2-Step Self-Etch	Kuraray	µSBS	#600 Silicon Carbide Paper	40.6 (± 4.7)
			Optibond Solo Self-Etch- Primer	2-Step Self-Etch	Kerr			36.2 (± 4.0)
			Tyrian SPE and One Step Plus–TY	2-Step Self-Etch	Bisco			24.1 (± 3.7)
			Adper Single Bond	2-Step Etch-and-Rinse	3M ESPE			41.5 (± 2.6)
			Scotchbond Multipurpose Plus	2-Step Etch-and-Rinse	3M ESPE			42.6 (± 5.5)
Uekusa *et al.*	34	2006	Clearfil tri-S Bond	1-Step Self-Etch	Kuraray	µTBS	#600 Silicon Carbide Paper	54.3 (± 9.0)
			One-Up Bond F	1-Step Self-Etch	Tokuyama			50.0 (± 8.7)

Table 1 *continued*

Author	Ref.	Year	Adhesive material	Classifications	Manufacturer	Test method (MPa)	Specimen staging method (dentine surface preparation)	Bond strength reduction (means ± SD)
Umino *et al.*	35	2006	Absolute	1-Step Self-Etch	Dentsply	µTBS	#600 Silicon Carbide Paper	27.6 (± 7.6) (Wet) 13.6 (± 8.6) (Dry) 24.8 (± 9.6) (Mix)
Pangsrisomboon *et al.*	36	2007	Clearfil SE Bond	2-Step Self-Etch	Kuraray	µTBS	#600 Silicon Carbide Paper	43.6 (± 9.2)
			One-up bond F	2-Step Self-Etch	Tokuyama			36.3 (± 6.5)
			Xeno III	1-Step Self-Etch	Dentsply			25.3 (± 8.0)
Proença *et al.*	37	2007	Clearfil SE Bond	2-Step Self-Etch	Kuraray	µTBS	#180 Silicon Carbide Paper	42.7 (± 10.7)
			Resulcin Aqua Prime	2-Step Self-Etch	Merz Dental			18.1 (± 6.8)
			One-up bond F	2-Step Self-Etch	Tokuyama			23.6 (± 11.1)
			Etch & prime 3.0	1-Step Self-Etch	Degussa AG			23.0 (± 9.6)
			Adper Prompt L-Pop	1-Step Self-Etch	3M ESPE			25.4 (± 18.9)
			Solist	1-Step Self-Etch	DMG GmbH			22.5 (± 9.7)
			Futurabond	1-Step Self-Etch	Voco GmbH			22.6 (± 11.5)
			Prime & Bond NT	2-Step Etch-and-Rinse	Dentsply			20.5 (± 7.7)
			Adper Single Bond	2-Step Etch-and-Rinse	3M ESPE			23.5 (± 4.4)
Albuquerque *et al.*	38	2008	Clearfil SE Bond	2-Step Self-Etch	Kuraray	µTBS	#600 Silicon Carbide Paper	18.0 (± 4.7)
			Adper Prompt L-Pop	1-Step Self-Etch	3M ESPE			24.3 (± 3.1)
			Xeno III	1-Step Self-Etch	DEntsply			30.0 (± 1.2)
			G Bond	1-Step Self-Etch	GC Corp			37.1 (± 2.4)
Marques *et al.*	39	2009	Clearfil SE Bond	2-Step Self-Etch	Kuraray	µTBS	#600 Silicon Carbide Paper	33.26 (± 9.59) SD – CAD
Scholtanus *et al.*	20	2010	Adper Scotchbond 1 XT	2-Step Etch-and-Rinse	3M ESPE	µTBS	#600 Silicon Carbide Paper	35 (± 10.6) – 25 (± 10.0)
			Clearfil S^3 Bond	1-Step Self-Etch	Kuraray			35 (± 8.5) – 21 (± 11.2)
			Clearfil SE Bond	2-Step Self-Etch	Kuraray			33 (± 9.2) – 39 (± 5.2)
Taschner *et al.*	40	2010	Adper Easy Bond	1-Step Self-Etch	3M ESPE	µTBS	#180 Silicon Carbide Paper	35.8 (± 5.7) (Etched) 26.9 (± 6.2) (Not etched)
			iBond Self-Etch	1-Step Self-Etch	Heraeus			24.3 (± 7.9) (Etched) 17.6c (± 4.3) (Not etched)
Belli *et al.*	41	2011	Adper Easy Bond	1-Step Self-Etch	3M ESPE	µTBS	#600 Silicon Carbide Paper	48.8 (± 15.5)
			Clearfil S^3 Bond	1-Step Self-Etch	Kuraray			31.8 (± 12.2)
			Clearfil SE Bond	2-Step Self-Etch	Kuraray			58.3 (± 17.7)
			Scotchbond Multipurpose	2-Step Etch-and-Rinse	3M ESPE			72.2 (± 18.2)
Toledano *et al.*	42	2012	Adper Single Bond	2-Step Etch-and-Rinse	3M ESPE	µTBS	#180 Silicon Carbide Paper	36.8 (± 9.9)
			Clearfil SE Bond	2-Step Self-Etch	Kuraray			39.7 (± 9.8)
			FL-Bond II	2-Step Self-Etch	Shofu			25.3 (± 5.3)
Mahdan *et al.*	43	2013	Bond Force	1-Step Self-Etch	Tokuyama	µTBS	#180 Silicon Carbide Paper	33.4 (± 9.7)
							#600 Silicon Carbide Paper	39.6 (± 8.3)
			Xeno V	1-Step Self-Etch	Dentsply		#180 Silicon Carbide Paper	32.7 (± 6.8)
							#600 Silicon Carbide Paper	37.0 (± 9.5)
			Clearfil S3 Bond	1-Step Self-Etch	Kuraray		#180 Silicon Carbide Paper	34.8 (± 7.9)
							#600 Silicon Carbide Paper	37.3 (± 9.8)
			Beautibond Multi	1-Step Self-Etch	Shofu		#180 Silicon Carbide Paper	34.9 (± 8.4)
							#600 Silicon Carbide Paper	38.9 (± 9.5)

to caries affected dentine papers were registered: authors, years, adhesive material, classification, manufacturer, test method, bond strength to sound and caries affected dentine expressed in MPa (Table 2).[18,20,21,44–67] For the MMPs effect on bond stability were evaluated: authors, years, adhesive material, classification, manufacturer, type of ageing, MMPs inhibitor and inhibitor use method (Table 3).[21,68–70]

Quality assessment

All studies meeting the inclusion criteria then underwent validity assessment. Two examiners (RP and MM) read the papers independently. The qualities and relevance of each study were graded using a study-quality checklist. External validity, internal validity and study precision were analysed to obtain an overall assessment of quality. The assessment was used as a basis for the discussion between the two examiners to grade the studies. In the case of disagreement, all authors discussed the paper until a consensus was reached.

RESULTS

The electronic searches identified 320 studies. Figure 4 summarizes the paper selection procedure. A total of 181 studies were excluded following a review of titles, abstracts and full text. The final analysis included 139 articles that conformed to the criteria for the present review.

Smear layer and affected dentine

Forty-six studies reported the smear layer effect on bond strength; 5 dealt with the ultrastructural appearance of the smear layer; 26 evaluated the effect of the manner in which the smear layer is created and 47 considered the interaction of adhesive systems and the smear layer on CAD. The cutting of dentine creates a layer of smear debris, which completely covers the surfaces and plugs the orifices of the dentinal tubules.[71]

The thickness, density and attachment of the smear layer to the underlying dentine is related to the way the smear layer was created, while its composition has the characteristics of the tissue which was cut.[29]

Generally speaking, the smear layer in dentine is basically formed by hydroxyapatite (HAP) and altered denatured collagen and, because of the inherent weakness, it can interfere with good adhesion.[72] This assumption was derived from the observation that when the smear was removed by etching there was better adhesion performance.[24] However, interferences might be directly related to the manner of smear creation (Table 1). Watanabe *et al*. demonstrated that a 'rough' or 'coarse' smear layer prepared by #180- or #400-grit abrasive papers remained relatively weak, even when impregnated by resins, suggesting the need to remove it from dentine.[72] Conversely, Toida *et al*. showed that the smear layer created by the use of burs was rougher than that formed by abrasive papers and more continuous with the underling dentine surface.[73] Thus, this type of smear layer might not interfere with the final quality of bonding in dentine if adequately infiltrated by the monomers.[72] However, Oliveira *et al*.[29] reported that carbide burs could create the smoothest surface but the weakest bond strength because of the characteristics of the smear. Spencer *et al*.,[74] using TEM and micro-RAMAN spectroscopy images, described this smear as a fibrous layer, composed of well-arranged and undisrupted collagen fibrils that might not be as easily dissolved by phosphoric acid or acidic monomers and so interfering with the permeation of bonding resin.

The composition of the smear layer in CAD has different aspects and chemical characteristics compared to that of unaltered dentine because of the different mineral/organic composition.[29] A CAD smear layer is richer in organic components and appears thicker than that of sound dentine.[75] Also, the collagen component is highly disorganized, traps minerals and can be difficult to remove even when acid-etching is used by etch-and-rinse adhesives (ERAs).[76] A greater amount of residue, compared with the etched sound dentine, may remain on the surface of CAD in a form of 'collagen smear layer' because acids only solubilize the mineral component of the smear layer.[71] This collagen smear layer is impermeable by the monomers and may impede homogeneous infiltration of the underlying dentine, affecting the quality of bonding, which finally derives from the homogeneity of strengthening in the demineralized dentine.[77,78] Poor infiltration of demineralized collagen may be connected to degradation of hybrid layers over time due to activation of the MMPs with hydrolysis of the non-reinforced fibrils.[79]

As in the case of ERAs, the smear layer might adversely affect the homogeneous hybrid layer when self-etching adhesives (SEAs) are used. SEA hybridization is formed by infiltration of the water rich channels of the smear layer reaching the partially demineralized superficial dentine, thus including the smear in the hybrid interdiffusion (Fig. 5).[80] However, thick smear layers might compromise superficial demineralization and reinforcement of collagen via early neutralization of acidic primers by the dentine buffering components of the smear.[29] SEAs with mild pHs could be less effective in infiltration of thick smear layers than those with lower pHs.[81] However, in this case more calcium-phosphate is dissolved in intertubular dentine compared with a mild acidic

Table 2. Systematic review of bond strength to caries-affected dentine papers

Author	Ref.	Year	Adhesive material	Classifications	Manufacturer	Test method (MPa)	Normal dentine (means ± SD)	*P*-value	Caries-affected dentine (means ± SD)
Nakajima *et al.*	18	1995	All Bond 2	3-Step Etch-and-Rinse	Bisco	μTBS	26.90 (± 8.83)	<0.05	13.01 (± 3.64)
			Scotchbond Multi-Purpose	3-Step Etch-and-Rinse	3M ESPE		20.32 (± 5.5)	NS	18.49 (± 4.04)
			Clearfil Liner Bond 2	2-Step Self-Etch	Kuraray		29.52 (± 10.90)	<0.05	13.97 (± 4.30)
Nakajima *et al.*	44	1999	Scotchbond Multi-Purpose	3-Step Etch-and-Rinse	3M ESPE	μTBS	42.4 (± 9.0)	NS	48.2 (± 3.9)
Nakajima *et al.*	45	1999	Clearfil Liner Bond 2	2-Step Self-Etch	Kuraray	μTBS	45.2 (± 13.9)	<0.05	29.7 (± 10.3)
			Clearfil Liner Bond 2v	2-Step Self-Etch	Kuraray		57.4 (± 10.4)	<0.05	39.1 (± 8.9)
			ART Bond	2-Step Self-Etch	Coltene		24.9 (± 17.5)	NS	30.2 (± 13.4)
Yoshiyama *et al.*	46	2000	Adper Single bond	2-Step Etch-and-Rinse	3M ESPE	μTBS	46.0 (± 10.5) (moist)	<0.05	27.1 (± 6.5) (moist)
							26.4 (± 4.8) (dry)	<0.05	18.1 (± 2.1) (dry)
			FluroBond	2-Step Self-Etch	FB Shofu		28.2 (± 6.11)	<0.05	17.5 (± 2.1)
Yoshiyama *et al.*	47	2002	ABF System	2-Step Self-Etch	Kuraray	μTBS	44.9 (± 14.6)	>0.05	50.9 (± 3.9)
			Adper Single Bond	2-Step Etch-and-Rinse	3M ESPE		25.3 (± 5.0)	>0.05	28.8 (± 6.3)
Ceballos *et al.*	48	2003	Prime & Bond NT	2-Step Etch-and-Rinse	Dentsply	μTBS	56.3 (± 11.1)	<0.05	41.3 (± 10.7)
			Scotchbond 1	2-Step Etch-and-Rinse	3M ESPE		43.9 (± 11.4)	NS	36.3 (± 12.2)
			Clearfil SE Bond	2-Step Self-Etch	Kuraray		35.5 (± 11.6)	<0.05	21.5 (± 5.5)
			Adper Prompt L-Pop	1-Step Self-Etch	3M ESPE		18.2 (± 9.6)	NS	13.4 (± 1.9)
Yoshiyama *et al.*	49	2003	Clearfil Liner Bond 2V	2-Step Self-Etch	Kuraray	μTBS	45 (± 10)	<0.05	30 (± 10)
Arrais *et al.*	50	2004	Clearfil SE Bond	2-Step Self-Etch	Kuraray	μTBS	41.82 (± 10.05) (Instructions)	<0.05	23.06 (± 7.84) (Instructions)
							48.70 (± 9.93) (Add. etching)	<0.05	30.76 (± 8.16) (Add. etching)
			Adper Single Bond	2-Step Etch-and-Rinse	3M ESPE		50.69 (± 10.81) (Instructions)	<0.05	23.58 (± 9.18) (Instructions)
							43.74 (± 8.97) (Add. etching)	<0.05	33.97 (± 12.18) (Add. etching)
Doi *et al.*	51	2004	Clearfil SE Bond	2-Step Self-Etch	Kuraray	μTBS	41.2 (± 10.0)	<0.05	13.2 (± 5.1)
			Mac-Bond II	2-Step Self-Etch	Tokuyama		35.0 (± 8.9)	<0.05	10.4 (± 1.6)
			Unifil Bond	2-Step Self-Etch	GC		27.2 (± 3.9)	<0.05	14.1 (± 4.5)
Yazici *et al.*	52	2004	Clearfil SE Bond	2-Step Self-Etch	Kuraray	μTBS	32.9 (± 13.7) (Instructions)	>0.05	15.9 (± 7.0) (Instructions)
							19.2 (± 5.8) (Add. etching)	>0.05	16.3 (± 5.7) (Add. etching)
Yoshiyama *et al.*	53	2004	ABF System	2-Step Self-Etch	Kuraray	μTBS	44.9 (± 14.6)	<0.05	25.5 (± 5.0)
Nakajima *et al.*	54	2005	Clearfil Protect Bond	2-Step Self-Etch	Kuraray	μTBS	43.5 (± 11.1)	<0.05	29.4 (± 7.5)
Say *et al.*	55	2005	Optibond Solo Plus	3-Step Etch-and-Rinse	Kerr	μTBS	38.7 (± 8.9) (Add. etching)	<0.05	28.5 (± 5.0) (Add. etching)
							44.2 (± 7.7) (+ OSP SE Primer)	<0.05	29.2 (± 4.3) (+ OSP SE Primer)
			Optibond Solo Plus + Activactor				7.2 (± 4.7) (Add. etching)	<0.05	10.5 (± 3.9) (Add. etching)
							18.3 (± 6.1) (+ OSP SE Primer)	<0.05	13.5 (± 3.3) (+ OSP SE Primer)
Sonoda *et al.*	56	2005	ABF System	2-Step Self-Etch	Kuraray	μTBS	32.36 (± 5.26)	<0.05	22.33 (± 6.90)
			Prime & Bond NT	2-Step Etch-and-Rinse	Dentsply		28.16 (± 16.31)	<0.05	25.06 (± 10.16)
Pereira *et al.*	57	2006	Single Bond	2-Step Etch-and-Rinse	3M ESPE	μTBS	52.0 (± 17.5)	>0.05	37.3 (± 9.7)
			Adper Prompt L-Pop	1-Step Self-Etch	3M ESPE		43.3 (± 14.1)	>0.05	36.1 (± 8.2)
Omar *et al.*	58	2007	Scotchbond Multipurpose	3-Step Etch-and-Rinse	3M ESPE	μTBS	22.19 (± 4.6)	<0.01	18.6 (± 2.89)
			Clearfil SE	2-Step Self-Etch	Kuraray		24.25 (± 5.7)	<0.01	20.7 (± 5.5)
			Xeno IV	1-Step Self-Etch	Dentsply		21.43 (± 7.6)	<0.01	15.45 (± 6.62)

Table 2 *continued*

Author	Ref.	Year	Adhesive material	Classifications	Manufacturer	Test method (MPa)	Normal dentine (means ± SD)	*P*-value	Caries-affected dentine (means ± SD)
Erhardt *et al.*	21	2008	Adper Scotchbond 1	2-Step Etch-and-Rinse	3M ESPE	µTBS	42.6 (± 6.2)	>0.05	34.5 (± 6.8)
			Clearfil Protect Bond	2-Step Self-Etch	Kuraray		39.2 (± 5.2)	>0.05	24.2 (± 7.0)
			AdheSE	1-Step Self-Etch	Ivoclar Vivadent		28.2 (± 6.3)	>0.05	17.2 (± 5.1)
Erhardt *et al.*	9	2008	Excite	2-Step Etch-and-Rinse	Ivoclar Vivadent	µTBS	25.6 (6.0)	<0.05	16.3 (5.2)
			Prime & Bond NT	2-Step Etch-and-Rinse	Dentsply		20.8 (3.2)	<0.05	16.0 (2.3)
Schiltanus *et al.*	20	2010	Adper Scotchbond 1XT	2-Step Etch-and-Rinse	3M ESPE	µTBS	35 (± 10.6)	>0.05	25 (± 10.0)
			Clearfil Tri-S Bond	1-Step Self-Etch	Kuraray		35 (± 8.5)		21 (± 11.2)
			Clearfil SE Bond	2-Step Self-Etch	Kuraray		33 (± 9.2)		39 (± 5.2)
Xuan *et al.*	60	2010	Adper Single Bond 2	2-Step Etch-and-Rinse	3M ESPE	µTBS	32.44 (± 5.59)	>0.05	28.98 (± 5.44)
			Clearfil SE Bond	2-Step Self-Etch	Kuraray		35.41 (± 5.62)	<0.01	21.18 (± 4.96)
			iBond GI	1-Step Self-Etch	Heraeus Kulzer		20.77 (± 4.73)	<0.01	14.50 (± 3.37)
Zanchi *et al.*	61	2010	Prime & Bond NT	2-Step Etch-and-Rinse	Dentsply	µTBS	38.63 (± 8.6)	<0.05	23.55 (± 5.4)
			Adper Single Bond 2	2-Step Etch-and-Rinse	3M ESPE		33.73 (± 12.6)		15.05 (± 5.3)
Zanchi *et al.*	62	2010	Adper Single Bond 2	2-Step Etch-and-Rinse	3M ESPE	µTBS	47.51 11.0 (Instructions)	<0.05	26.64 10.3 (Instructions)
							40.20 10.2 (Add. etching 15”)	<0.05	33.43 11.9 (Add. etching 15”)
							35.36 12.7 (Add. etching 30”)	<0.05	35.29 12.0 (Add. etching 30”)
			Clearfil SE Bond	2-Step Self-Etch	Kuraray		42.24 8.3 (Instructions)	<0.05	23.02 7.1 (Instructions)
							46.59 9.9 (Add. etching 15”)	<0.05	29.31 9.1 (Add. etching 15”)
							51.28 8.5 (Add. etching 30”)	<0.05	34.18 10.6 (Add. etching 30”)
Aggarwal *et al.*	63	2011	Adper Easy One	1-Step Self-Etch	3M ESPE	µTBS	23.42 (± 3.39)		17.42 (± 2.32)
			Adper Single Bond	2-Step Etch-and-Rinse	3M ESPE		27.86 (± 3.45)		22.90 (± 3.44)
Mobarak *et al.*	64	2012	Clearfil SE Bond	2-Step Self-Etch	Kuraray	µSBS	22.34 (± 6.4)	>0.05	18.70 (± 4.09)
			Clearfil DC Bond	1-Step Self-Etch	Kuraray		24.49 (± 8.0)	>0.05	18.97 (± 9.40)
			Bond Force	1-Step Self-Etch	Tokuyama		24.52 (± 4.9)	<0.05	18.31 (± 4.90)
			AdheSE One	1-Step Self-Etch	Ivoclar		17.21 (± 6.8)	>0.05	17.31 (± 10.3)
			Adper Prompt-L-Pop	1-Step Self-Etch	3M ESPE		13.67 (± 4.4)	<0.05	7.31 (2± .40)
Alves *et al.*	65	2013	Adper Single Bond	2-Step Etch-and-Rinse	3M ESPE	µTBS	35.5 (± 3.5)	<0.05	17.8 (± 4.2)
			Adper SE Plus	1-Step Self-Etch	3M ESPE		18.2 (± 6.5)	<0.05	13.9 (± 3.2)
			Adper Easy Bond	1-Step Self-Etch	3M ESPE		26.3 (± 1.9)	<0.05	14.4 (± 4.2)
Joves *et al.*	66	2013	Clearfil SE Bond	2-Step Self-Etch	Kuraray	µTBS	80.8 (± 18.0)	>0.05	37.6 (± 14.3)
			Clearfil Protect Bond	2-Step Self-Etch	Kuraray		62.0 (± 12.6)		34.6 (± 9.9)
Erhardt *et al.*	67	2014	Adper Single Bond	2-Step Etch-and-Rinse	3M ESPE	µTBS	31.0 (± 4.3)	<0.05	24.0 (± 5.9)
			Clearfil SE Bond	2-Step Self-Etch	Kuraray		28.0 (± 6.0)	<0.05	19.3 (± 6.7)

Table 3. Systematic review of the MMPs effect on bond stability to caries-affected dentine papers

Author	Ref.	Year	Adhesive material	Classifications	Manufacturer	Type of ageing	Test method (MPa)	MMP inhibitor	Inhibitor use method	Bond strength reduction (means ± SD)			
										24 hours SD	CAD		
Erhardt *et al.*	21	2008	Adper Scotchbond 1	2-Step Etch-and-Rinse	3M ESPE	Water stored (distilled water)	µTBS	EDTA° CHX* 0.05% Control	Pretreatment solution	28.1 (± 4.2) 27.3 (± 4.2) 31.9 (± 3.3)	24.9 (± 5.8) 24.9 (± 5.8) 26.2 (± 3.5)		
										24 hours SD	CAD	24 months SD	CAD
Mobarak	68	2011	Clearfil SE Bond	2-Step Self-Etch	Kuraray	Artificial saliva	µTBS	CHX* 0.02%	Pretreatment solution	23.7 (± 5.9)	20.8 (± 6.2)	8.7 (± 3.2)	9.9 (± 3.4)
								CHX* 0.05% Control		25.9 (± 6.4) 24.3 (± 5.1)	20.5 (± 5.1) 21.7 (± 6.0)	10.9 (± 3.3) 9.4 (± 3.4)	14.6 (± 4.5) 9.9 (± 3.5)
										24 hours SD	CAD		
Lenzi *et al.*	69	2012	Adper Single Bond 2	2-Step Etch-and-Rinse	3M ESPE	Water stored (distilled water)	µTBS	CHX* 0.02% Control	Pretreatment solution	43.2 (± 4.7) 41.7 (± 2.7)	36.4 (± 1.3) 29.1 (± 6.0)		
										24 hours SD	CAD	12 months SD	CAD
Ekambaram *et al.*	70	2014	Ethanol-wet bonding	3-Step Etch-and-Rinse	Experimental material	Artificial saliva	µTBS	CHX* 0.02% Control	Pretreatment solution	51.7 (± 3.9) 52.9 (4.4)	40.8 (± 5.7) 50.3 (4.1)	33.6 (± 4.2) 33.9 (3.3)	28.7 (± 3.6) 31.7 (5.2)
			Water-wet bonding	3-Step Etch-and-Rinse	Experimental material			CHX* 0.02% Control		25.2 (± 4.1) 26.6 (3.8)	18.2 (± 4.0) 26.5 (1.9)	20.5 (3.6) 23.4 (4.8)	8.0 (3.1) (19.0 (3.5)

°Ethylenediaminetetraacetic acid.
*Chlorhexidine.

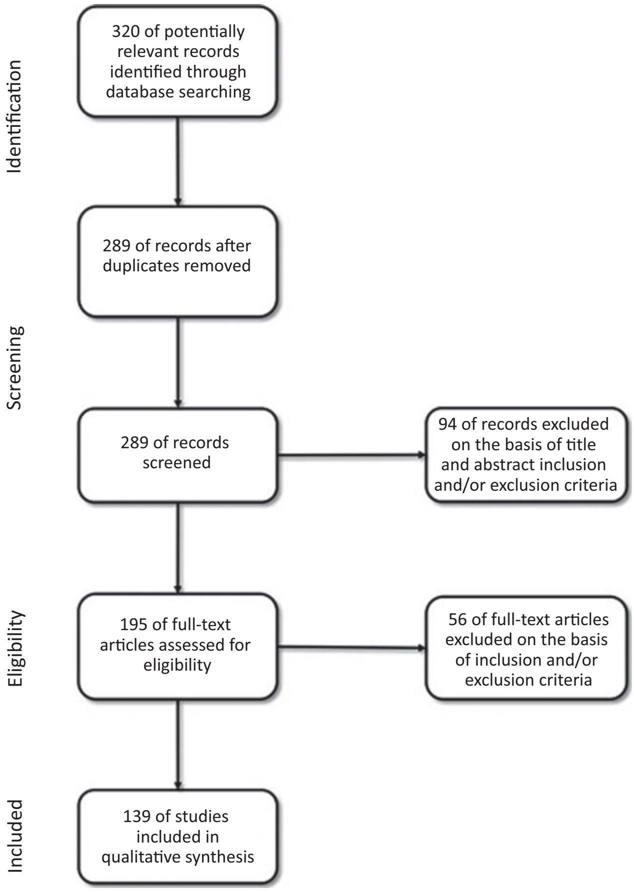

Fig. 4 Search flowchart according to PRISMA Statement.

primer.[82,83] Consequently, an increase in wetness and porosities has to be considered in collagen using strong SEAs, which have difficulty in being infiltrated by hydrophobic adhesive resins.[84,85]

Nevertheless, as far as we are aware, smear layer interferences remain a controversial issue in CAD, as well as in sound dentine regardless of the adhesive approach (Table 2).[20] Some studies reported low dentine bond strengths over thick dentine smear layers,[24,25] while others reported no influence in strength,[27,81] even if using mild SEAs,[84] particularly in the early bond strength values.[33] Considering the long-term values, hydrolytic degradation of polymers after water sorption, together with permeability of adhesive layers are likely to be considered as the main causes of low bonding.[85,86]

ERA bonding and affected dentine

Twenty-one selected papers were evaluated regarding the morphological and chemical interaction of ERAs in CAD.

Bonding by ERAs consists of a first phase of etching and rinsing, followed by infiltration of adhesive monomers in the demineralized surface. The result is a mixture of inorganic resin monomers and organic demineralized dental tissue in the form of a hybrid layer with resin tags in the tubules (Fig. 6).[71,87,88]

Although in CAD the hybrid layer of ERAs is thicker than in sound dentine, the reinforcement of intertubular collagen and tubular tags may be compromised.[89] Many factors may interfere with a tight bond in CAD using ERAs. The soft, already demineralized collagen,[90] the high degree of porosity and wetness,[46] a lack of minerals around and within the fibrils,[16] as well as spectral changes in the secondary structure of the collagen,[8] may cause much aggressive etching in CAD.[91] Consequently, etching CAD may result in too much demineralization[92] compared with the concentration gradient of monomer infiltration and discrepancies in reinforcement at the bottom of the hybrid layer.[92] There is also the possibility of increasing wetness, as the removal of calcium takes up additional water in the tissue and more water may already be present due to the rinsing phase of the acid.[93] It has been shown that water is helpful in keeping the demineralized interfibrillar channels physically expanded, allowing monomer percolation.[71] However, at the same time, water may produce: (1) a lower degree of resin monomer conversion;[8,94]

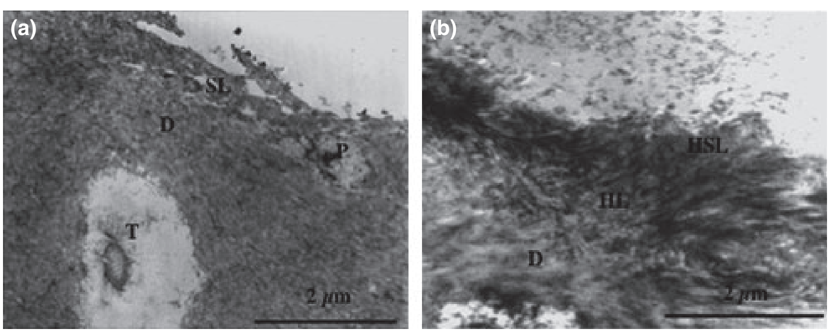

Fig. 5 In Panel a, TEM photomicrographs of a CAD surface covered by a porous smear layer (SL) of degenerated collagen fibrils with trapped crystallites. At the dentine front, a tubule appears smear plugged (P) while the other (T) is occluded by crystallites of different electron densities. Panel b shows a mild self-etching hybrid layer (HL), Clearfil SE Bond (Kuraray, Osaka, Japan). Hybridization of self-etchings is formed through infiltration of the water rich channels of smear layer reaching the partially demineralized superficial dentine (D), thus including the smear in the uppermost part of hybridization (HSL).

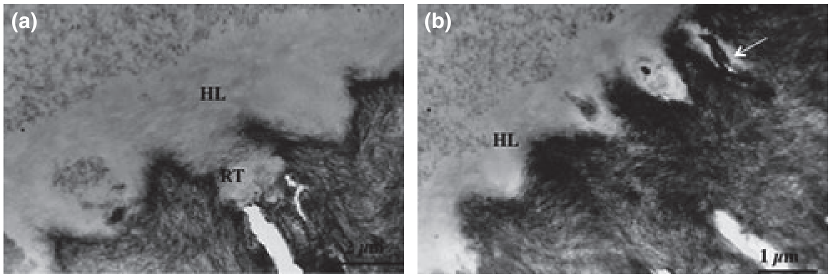

Fig. 6 Non-demineralized, unstained TEM images of hybrid layers formed by an etch-and-rinse adhesive (OptiBond Solo, Kerr Corporation, Orange, CA, USA). Etch-and-rinse adhesives completely deprive the tissue surface of smear layer because of the etching procedure. The hybrid layer is primarily based on a diffusion process into the totally demineralized collagen, and micromechanical interlocking in tubules by resin tags. In Panel a, UD, the hybrid interdiffusion appears reinforced by long resin tags (RT), while in CAD (Panel b), tubular mineral occlusions (TM) not dissolved by the etching, impede the formation of the tubular tags.

(2) interference with the reinforcement of the hydrophobic Bis-GMA adhesives;[10] and (3) phase separations between the hydrophobic and hydrophilic components of adhesives.[95] All of these factors may result in non-homogeneity and porosities at the interface as an expression of suboptimal sealing in CAD.[21,96,97]

To reduce interference by water, evaporation of the water rinsing in CAD as well as in sound dentine is favoured using air drying.[71] However, it may shrink the demineralized collagen fibrils, narrowing the inter-fibrilar channels[98] and rendering impossible infiltration by the monomers. As a result, bond strength would be limited to the strength of surface adhesion,[71] leaving behind exposed and non-reinforced fibrils.

In regard to a tubular occlusion, the use of strong acid cannot lead to dissolution of intratubular minerals, thus affecting percolation of resins and resin tag formations.[46] At the same time, the low buffer capacity of the minerals may allow high demineralization and wetness in peritubular dentine with residual porosities in interdiffusion.[10]

These considerations might explain a higher susceptibility of the affected interface, in comparison with sound dentine, to acid and base treatments, with degradation phenomena of CAD hybrid layers.[75,99]

SEA bonding and affected dentine

Thirty-four selected papers evaluated the morphological and chemical interaction of SEAs in CAD.

SEAs avoid the separate etching phase of ERAs due to the presence of acidic functional monomers in their chemistry. Thus, functional monomers demineralize and infiltrate the tissue at the same time.

In the case of 'Two-Step SEAs', SEA hybridization is created in two procedures; the first of which is the application of a primer of different pH acidity, followed by the use of an adhesive resin, generally Bis-GMA based (Fig. 7). In the 'One-Step SEAs', acidic and adhesive monomers are mixed in the same bottle, thereby causing hybridization at the same time.

In both cases, functional monomers have the capacity to interact with HAP and collagen by a series of chemical atomic-level interactions with an advantage in tissue strength.[82,100] The interaction of 10-MDP (10-Methacryloyloxydecyl dihydrogen phosphate) mild functional monomer (pH = 2) has shown better bonding and durability compared with the strong 4-MET (4-methacryloxyethyl trimellitic acid) and phenil-P.[83,101] This different behaviour was explained by the mode of interaction of the functional

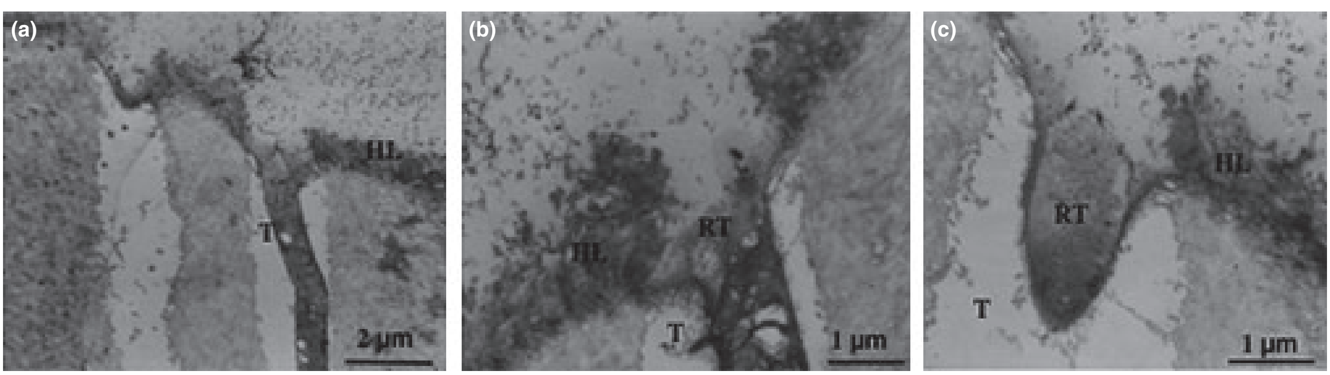

Fig. 7 TEM of the dentine interdiffusion of Clearfil Protect Bond (Kuraray, Osaka, Japan), a 10-MDP mild self-etching, in UD. The MDP functional monomer in the primer allows for partial demineralization of the collagen with exposure of hydroxyapatite. Additionally, the MDP-primer interacts with hydroxyapatite and collagen matrix phases with a series of chemical atomic-level interactions and a strong chemical bond. In Panels a and b, the hybrid layer (HL) is formed by an irregular top of infiltered smear layer with short resin tags (RT) in the tubules (T), clearly discernible in (c).

monomers, which is inversely related to their acidity. 10-MDP SEAs cause a regularly layered structure on the surface, within which more highly insoluble calcium salts are deposited.[102] This means that mild SEAs could be more effective in CAD compared with strong ones, as a mild acidity primer is able to keep HAP crystals attached around the demineralized collagen, preventing fibrils from being exposed and hydrolysed in environmental fluids (Fig. 8 and 9).[12,97,103–108]

The low acidity of strong SEAs completely deprives the fibrils of HAP, thus creating a calcium-depleted hybrid layer through a primarily diffusion-based mechanism, as in ERAs (Fig. 10). Moreover, the strong SEA interdiffusion retains unstable calcium-phosphate salts.[109] Additionally, the low pH cannot dissolve the mineral deposits in the dentinal tubules,[71,110] in which resin tags will not be formed. As in the case of ERAs, a primer with a low acidity may raise dissolution and wetness in peritubular areas[71] with problems related to the saturation of the hydrophobic Bis-GMA resin. Suboptimal infiltration could explain a gradual decrease in bond strength in CAD under oral stress simulation, which may be more relevant to the global strength than the absence of resin tags in the mineralized tubules.[46] Also, non-homogeneous reinforcement may lower the bonding significantly after six months of water exposure,[21] via a possibility of nanoleakage along the hybrid layer of some SEAs.[105,111]

Important considerations concern an inhibitory effect on secondary caries using SEAs in CAD.[112] An electron dense zone was reported underlining the hybrid layer formed by 10-MDP containing SEAs after exposure to an artificial demineralizing solution (pH 4.5) for 90 minutes and then 5% sodium hypochlorite for 20 minutes. This area was identified as an 'acid-base resistant zone'.[112] Morphologically, the acid-base resistant zone showed densely packed crystallites, probably formed by resin-infiltrated dentine. This suggested that some chemical reactions might take place between HAP and 10-MDP in dentine, with the effect of increasing the resistance to acid attacks of microorganisms and thus secondary caries.

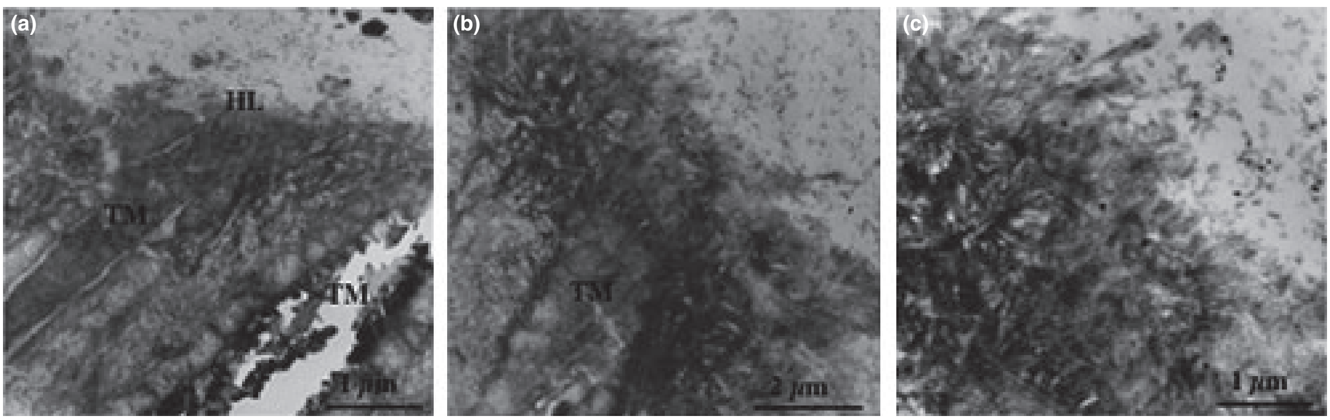

Fig. 8 TEM observation of the hybrid layer of Clearfil Protect Bond (Kuraray, Osaka, Japan), in CAD. In Panels a and b, the hybrid layer (HL) appears deprived of resin tags due to the presence of minerals in the tubules (TM). The mild acidity primer is able to keep HAP crystals attached around the already demineralized collagen (c), preventing fibrils from being exposed and hydrolysed in environmental fluids.

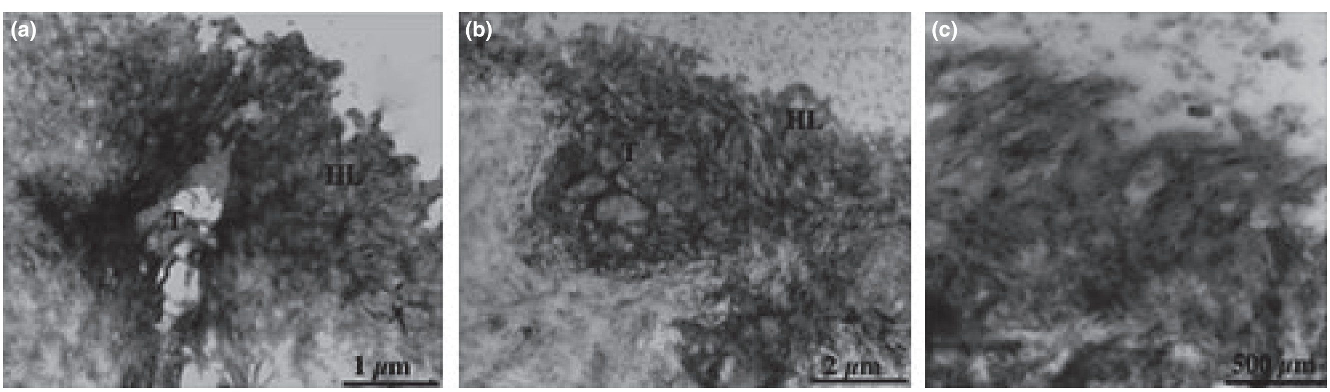

Fig. 9 TEM morphology of the hybrid layer formed by Clearfil SE Bond (Kuraray, Osaka, Japan), in CAD. In Panels a and b, an irregular and ruffled border of hybridized smear layer residue is evident at the top of the interdiffusion. Nevertheless, real resin tags cannot be formed in the mineralized tubules of the interface. A dense infiltration of the peritubular dentine toward the intertubular dentine can be observed. In (c), affected collagen fibrils retain dense crystallites owing to a good interaction of the mild functional monomer.

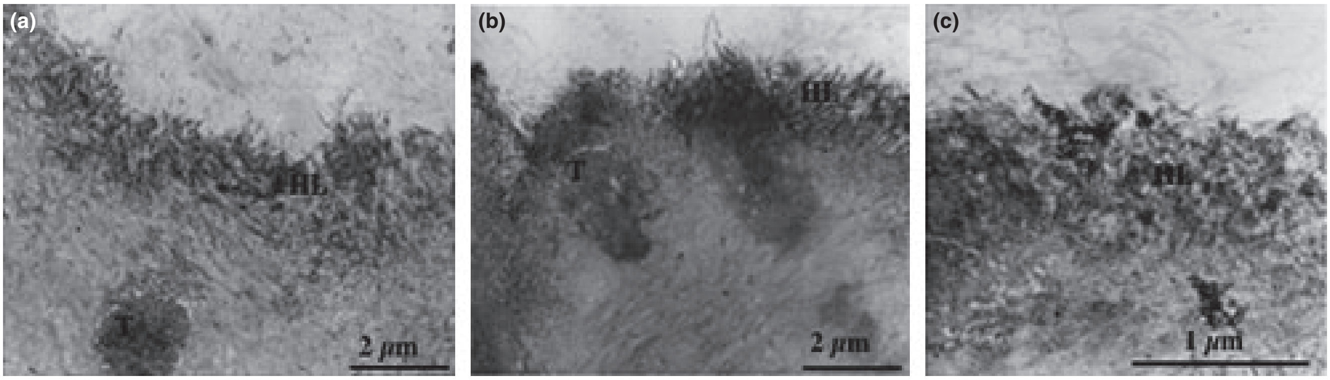

Fig. 10 In Panel a, the strong self-etching system Tyrian SPE-One Step Plus forms a hybrid layer (HL) completely deprived of smear layer and hydroxyapatite. The strong self-etching completely deprives the fibrils of HAP, thus creating a calcium-depleted hybrid layer through a primarily diffusion-based mechanism, as in etch-and-rinse adhesives. In Panel b, the dentinal tubules are obstructed by crystals, which are not affected by the low pH. In Panel C, the hybrid layer shows non-homogeneous reinforcement and residual porosities, which may result in instability of the interdiffusion and hydrolytic degradation over time.

In regard to the use of One-Step SEAs, they are complex mixtures of hydrophilic and hydrophobic components which acidify, prime and bond simultaneously.

These systems result in very thin hybrid layers, which are prone to less polymerization[113] and high permeation by fluids (Fig. 11).[11,114,115] This behaviour has been attributed to the incorporation of high concentrations of hydrophilic monomers, i.e. HEMA,[114,115] which allow the absorption of water from the dentine fluids towards the dentine interface.[116–118]

In vitro experimentations[119] reported that the mineral occlusion of CAD might prevent the permeation of water fluids in One-Step SEA interdiffusion. However, silver nitrate uptake, as well as adhesive/mixed fractures, were reported in the adhesive interface of OSA after water storage.[65] Also, in clinical conditions of pulpal pressure, OSA's hybrid layers have been shown to be permeable.[10] Very different permeability results were reported in CAD in clinical conditions, which were explained by the quality and quantity of dentine removed during excavation of carious tissue.[10]

Water sorption and permeability of these hybrid layers are likely to be the cause of hydrolysis in OSA.[120]

To optimize the composition of self-etching adhesives, just enough HEMA should be added to wet the dentine and prevent excessive water sorption, phase separations of dimethacrylates and solubility. However, such a compromise may not create optimal bonds.[116] Thus, acrylamide-based adhesive systems have been designed to overcome the problem of hydrolytic instability, promising a better performance of the latest generation of OSAs.[121]

MMPs and affected dentine

Thirty papers evaluated the effect of MMP inhibitors' ability to prevent hybrid layer instability. The specific

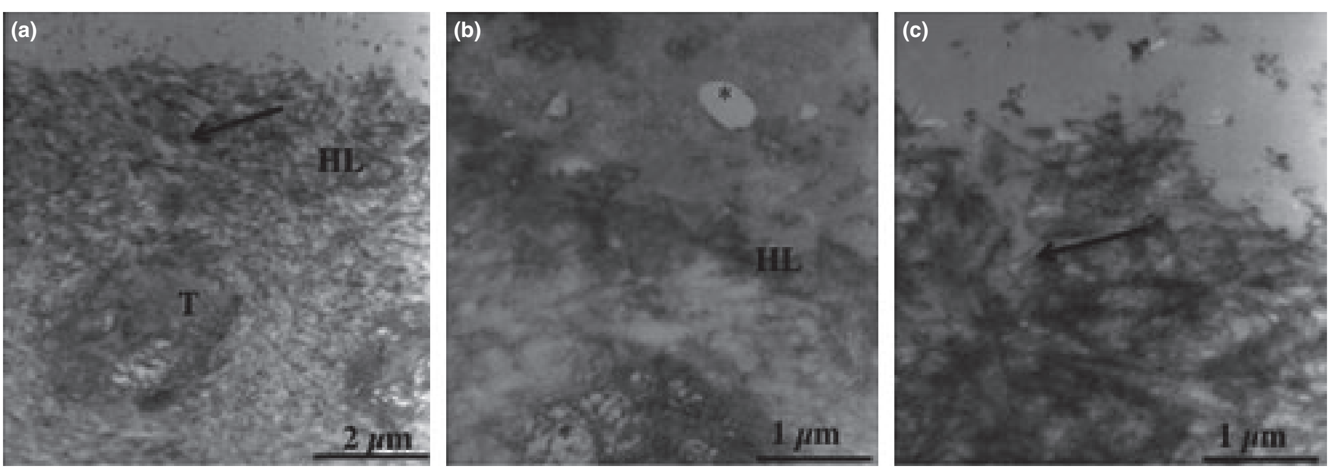

Fig. 11 TEM sections of the very thin hybrid layer formed by XENO 3 (Dentsply, Caulk, Germany) one-step self-etching system. The interdiffusion was made in CAD *in vivo* clinical conditions and pulpal pressure, and samples were extracted after 10 minutes. The high hydrophilic aspect of the hybrid layer is notable (a, b and c) and shows voids (asterisks), and channels of water (arrows) running towards the interface by means of the unsealed tubules. These common problems in the one-step self-etching systems are connected to the high concentrations of hydrophilic HEMA monomers and solvents.

morphology and environmental factors in CAD may allow enzymatic degradation of the hybrid layer through penetration of moisture in the polymer bulk.[114,122,123]

Hydrolysis of hybrid layers occurs because the demineralized, non-reinforced fibrils may undergo self-destruction due to activation of MMPs[21] and cathepsins enzymes, which are secreted in the form of pro-enzymes by the odontoblast.[5,124,125] MMPs need an acid microenvironment[6] in order to become active.[7] As in the case of the carious process, during which MMPs are activated by the cariogenic bacteria releasing of lactate, proteolytic enzymes are able to degrade demineralized, exposed collagen which is part of the chemical polymers[96] after acidic priming in bonding procedures.

MMPs may be activated by the acidic properties of adhesive systems,[124,125] as pH microenvironmental changes may alter the conformation of the propeptide in active form. Some studies have shown a correlation between the low pH of a primer and activation of the enzymes, even in the case of phosphoric acid etching, denaturing enzymes themselves or reducing their activity.[126]

Immunohistochemical studies revealed that MMPs can be stimulated by SEAs from the dentino-pulp complex and more precisely from odontoblast.[127]

Water is a necessary factor in the hydrolytic function of the enzymes. It is needed to hydrolyse peptide bonds in collagen, resulting in degradation of the resin-dentine interface.[128] Hydrolysis gives rise to a progressive decrease in mechanical properties and strengths of the hybrid layer.[120] The importance of water has been evidenced by studies demonstrating no loss of dentine bonding over time when mineral oil was used as a storage medium instead of water.[129]

MMP inhibitors should be recommended to antagonize the hydrolysis of hybrid layers. The use of MMP inhibitors may cause the breakdown of dentine collagen and, at the same time, allow undisturbed remineralization when CAD is bonded (Table 3). With this purpose in mind, different agents and methods have been proposed to treat dentine after an acid priming: (1) calcium and zinc chelators from acid-etched dentine, as the presence of calcium and zinc ions are necessary to MMPs to became activated;[130,131] (2) protein cross-linking agents to cross-link their peptide chains immediately after acid-etching;[132] (3) specific versus non-specific inhibitors of proteases added directly to primers.[124,132,132–135]

Also, the ethanol-wet bonding technique has been shown to be a method which prevents hydrolysis. Ethanol is used as a solvated primer to chemically dehydrate acid-etched demineralized dentine.[136,137] This results in shrinkage of collagen with a consequent increase in the interfibrillar spaces, which may be easily infiltrated by monomers. At the same time, the reduced hydrophilicity of collagen matrix allows fibrils to be densely covered by resin, keeping them free of water uptake.

Recently the use of chlorhexidine has been shown to be a more suitable agent as a MMP inhibitor, even at low concentrations.[138] Using chlorhexidine as an additional primer in ERAs, the collagen fibrils have shown the capacity to maintain their structural integrity[124] with an increase in strength after six months of water storage.[129] The mechanism of inhibition may derive from its zinc cation-chelating property.[138] Also, chlorhexidine is able to interact with the residual mineral phase of the dentine matrix after acid etching,[21] allowing binding to phosphate groups, the increased affinity for tooth surfaces after etching and augmenting the dentine free energy surface.

However, chlorhexidine in CAD, in the case of sound dentine, may be less effective. This derives from the fact that when etching is applied to CAD, the extrafibrillar mineral is completely dissolved and the intrafibrillar mineral is non-homogeneously distributed,[131] somewhat affecting the effectiveness of this solution.

In any case, even if chlorhexidine helps to preserve the structure and function of both sound and CAD hybrid layers, it is necessary to determine whether its effect is adhesive system specific, being dependent upon the composition of the applied adhesive resin.

CONCLUSIONS

Despite the great improvement in adhesion technology over recent decades, CAD bonding needs to be further understood and improved. Several aspects need to be clarified in order to enhance strength and durability. Morphological and chemical characteristics strongly influence the response of CAD in bonding which, regardless of the use of adhesives, demonstrates lower strength and durability than sound dentine. The loss of minerals, wetness and tubular occlusions may cause global decreases in bond strength and longevity of the CAD interface by activation of MMPs.

Etching procedures using ERAs are questionable in CAD. The characteristic composition of the CAD smear after etching may form a layer of residue on the surface that is quite impermeable to the monomer, causing non-homogeneous infiltration of CAD. Etching can also be too aggressive and deep in CAD, completely depriving the interfibrillar affected collagen of HAP reinforcement, and directly altering the conformation of the collagen. Consequently, etching may cause discrepancies between the depth of demineralization and reinforcement by the adhesives as well as cause permanent exposed fibrils at the deepest region of the hybrid layer, which are prone to be hydrolysed by the MMPs.

As in the case of ERAs, the smear layer might adversely affect the homogeneous hybrid layer when SEAs are used in CAD. Thick smear layers might affect superficial demineralization and reinforcement of collagen via early neutralization of the acidic primers in SEAs, particularly when using mild pH primers. At the same time, the mild SEAs could be more effective in CAD compared with strong ones, as a mild acidity primer is able to keep HAP crystals attached around the demineralized collagen, preventing fibrils from being exposed and hydrolysed in environmental fluids. As in the case of ERAs, strong SEAs may be too aggressive in CAD. Nanoleakage in the hybrid layer, as a consequence of poor resin saturation, is the basis of a progressive decrease in strength and also the basis of hydrolysis by reactivation of MMPs. This explains a slow disappearance of the hybrid layers by the digestion of the collagen matrix in the polymer bulk over time. The use of MMP inhibitors, chlorhexidine being the most creditable, is strongly suggested in CAD bonding, particularly when strong acidic primers are used.

REFERENCES

1. Manji F, Fejerskov O. Dental caries in developing countries in relation to the appropriate use of fluoride. J Dent Res 1990;69:733–741.
2. Manji F, Fejerskov O, Nagelkerke NJ, Baelum V. A random effect model for some epidemiological features of dental caries. Community Dent Oral Epidemiol 1991;19: 324–328.
3. Fusayama T. Two layers of carious dentin: diagnosis and treatment. Oper Dent 1979;4:63–70.
4. Marshall Jr GW, Marshall SJ, Kinney JH, Balooch M. The dentin substrate: structure and properties related to bonding. J Dent 1997;25:441–458.
5. Linde A. Dentin matrix proteins: composition and possible functions in calcification. Anat Rec 1989;224:154–166.
6. Chaussain-Miller C, Fioretti F, Goldberg M, Menashi S. The role of matrix metalloproteinases (MMPs) in human caries. J Dent Res 2006;85:22–32.
7. Fusayama T, Terachima S. Differentiation of two layers of carious dentin by staining. J Dent Res 1972;51:866.
8. Spencer P, Wang Y, Katz JL, Misra A. Physicochemical interactions at the dentin/adhesive interface using FTIR chemical imaging. J Biomed Opt 2005;10:031104.
9. Jensen AT, Hansen KG. Tetracalcium hydrogen triphosphate trihydrate a constituent of dental calculus. Experientia 1957;13:311.
10. Milia E, Pinna R, Castelli G, *et al*. TEM morphological characterization of a one-step self-etching system applied clinically to human caries-affected dentin and deep sound dentin. Am J Dent 2012;25:321–326.
11. Ito S, Hashimoto M, Wadgaonkar B, *et al*. Effects of resin hydrophilicity on water sorption and changes in modulus of elasticity. Biomaterials 2005;26:6449–6459.
12. 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.
13. Marshall GW, Habelitz S, Gallagher R, Balooch M, Balooch G, Marshall SJ. Nanomechanical properties of hydrated carious human dentin. J Dent Res 2001;80:1768–1771.
14. Angker L, Nockolds C, Swain MV, Kilpatrick N. Correlating the mechanical properties to the mineral content of carious dentine – a comparative study using an ultra-micro indentation system (UMIS) and SEM-BSE signals. Arch Oral Biol 2004;49:369–378.
15. Zheng L, Nakajima M, Higashi T, Foxton RM, Tagami J. Hardness and Young's modulus of transparent dentin associated with aging and carious disease. Dent Mater J 2005;24:648–653.
16. Kinney JH, Balooch M, Marshall GW, Marshall SJ. A micromechanics model of the elastic properties of human dentine. Arch Oral Biol 1999;44:813–822.
17. Wang Y, Spencer P, Walker MP. Chemical profile of adhesive/caries-affected dentin interfaces using Raman microspectroscopy. J Biomed Mater Res 2007;81:279–286.
18. Nakajima M, Sano H, Burrow MF, *et al*. Tensile bond strength and SEM evaluation of caries-affected dentin using dentin adhesives. J Dent Res 1995;74:1679–1688.
19. Nakajima M, Hosaka K, Yamauti M, Foxton RM, Tagami J. Bonding durability of a self-etching primer system to normal and caries-affected dentin under hydrostatic pulpal pressure in vitro. Am J Dent 2006;19:147–150.
20. Scholtanus JD, Purwanta K, Dogan N, Kleverlaan CJ, Feilzer A. Microtensile bond strength of three simplified adhesive systems to caries-affected dentin. J Adhes Dent 2010;12:273–278.
21. Erhardt MC, Toledano M, Osorio R, Pimenta LA. Histomorphologic characterization and bond strength evaluation of caries-affected dentin/resin interfaces: effects of long-term water exposure. Dent Mater 2008;24:786–798.
22. Moher D, Liberati A, Tetzlaff J, Altman DG; PRISMA Group. Preferred reporting items for systematic reviews and meta-analyses: the PRISMA statement. J Clin Epidemiol 2009;62:1006–1012.
23. Frankenberger R, Perdigão J, Rosa BT, Lopes M. 'No-bottle' vs 'multi-bottle' dentin adhesives–a microtensile bond strength and morphological study. Dent Mater 2001;17:373–380.
24. Koibuchi H, Yasuda N, Nakabayashi N. Bonding to dentin with a self-etching primer: the effect of smear layers. Dent Mater 2001;17:122–126.
25. Ogata M, Harada N, Yamaguchi S, Nakajima M, Pereira PN, Tagami J. Effects of different burs on dentin bond strengths of self-etching primer bonding systems. Oper Dent 2001;26:375–382.
26. Chaves P, Giannini M, Ambrosano GM. Influence of smear layer pretreatments on bond strength to dentin. J Adhes Dent 2002;4:191–196.
27. 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.
28. Chan KM, Tay FR, King NM, Imazato S, Pashley DH. Bonding of mild self-etching primers/adhesives to dentin with thick smear layers. Am J Dent 2003;16:340–346.
29. Oliveira SS, Pugach MK, Hilton JF, Watanabe LG, Marshall SJ, Marshall GW Jr. The influence of the dentin smear layer on adhesion: a self-etching primer vs. a total-etch system. Dent Mater 2003;19:758–767.
30. Dias WR, Pereira PN, Swift EJ Jr. Effect of surface preparation on microtensile bond strength of three adhesive systems to bovine enamel. J Adhes Dent 2004;6:279–285.
31. Toledano M, Osorio R, Moreira MA, *et al*. Effect of the hydration status of the smear layer on the wettability and bond strength of a self-etching primer to dentin. Am J Dent 2004;17:310–314.
32. Kenshima S, Reis A, Uceda-Gomez N, *et al*. Effect of smear layer thickness and pH of self-etching adhesive systems on the bond strength and gap formation to dentin. J Adhes Dent 2005;7:117–126.

33. Reis A, Grandi V, Carlotto L, *et al*. Effect of smear layer thickness and acidity of self-etching solutions on early and long-term bond strength to dentin. J Dent 2005;33:549–559.
34. 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.
35. Umino A, Nikaido T, Sultana S, Ogata M, Tagami J. Effects of smear layer and surface moisture on dentin bond strength of a waterless all-in-one adhesive. Dent Mater J 2006;25: 332–338.
36. Pangsrisomboon B, Harnirattisai C, Nilsri K, Burrow MF. Microtensile bond strength of self-etching adhesive systems to differently prepared dentin. Am J Dent 2007;20:259–262.
37. Proença JP, Polido M, Osorio E, *et al*. Dentin regional bond strength of self-etch and total-etch adhesive systems. Dent Mater 2007;23:1542–1548.
38. Albuquerque M, Pegoraro M, Mattei G, Reis A, Loguercio AD. Effect of double-application or the application of a hydrophobic layer for improved efficacy of one-step self-etch systems in enamel and dentin. Oper Dent 2008;33:564–570.
39. Marques MS, Kenshima S, Muench A, Ballester RY, Rodrigues Filho LE. Effect of the C-factor and dentin preparation method in the bond strength of a mild self-etch adhesive. Oper Dent 2009;34:452–459.
40. Taschner M, Nato F, Mazzoni A, *et al*. Role of preliminary etching for one-step self-etch adhesives. Eur J Oral Sci 2010;118:517–524.
41. Belli R, Sartori N, Peruchi LD, *et al*. Effect of multiple coats of ultra-mild all-in-one adhesives on bond strength to dentin covered with two different smear layer thicknesses. J Adhes Dent 2011;13:507–516.
42. Toledano M, Cabello I, Yamauti M, Osorio R. Differential resin-dentin bonds created after caries removal with polymer burs. Microsc Microanal 2012;18:497–508.
43. Mahdan MH, Nakajima M, Foxton RM, Tagami J. Combined effect of smear layer characteristics and hydrostatic pulpal pressure on dentine bond strength of HEMA-free and HEMA-containing adhesives. J Dent 2013;41:861–871.
44. Nakajima M, Sano H, Zheng L, Tagami J, Pashley DH. Effect of moist vs. dry bonding to normal vs. caries-affected dentin with Scotchbond Multi-Purpose Plus. J Dent Res 1999;78:1298–1303.
45. 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;12:309–314.
46. Yoshiyama M, Urayama A, Kimochi T, Matsuo T, Pashley DH. Comparison of conventional vs self-etching adhesive bonds to caries-affected dentin. Oper Dent 2000;25:163–169.
47. Yoshiyama M, Tay FR, Doi J, *et al*. Bonding of self-etch and total-etch adhesives to carious dentin. J Dent Res 2002;81: 556–560.
48. Ceballos L, Camejo DG, Victoria Fuentes M, *et al*. Microtensile bond strength of total-etch and self-etching adhesives to caries-affected dentine. J Dent 2003;31:469–477.
49. Yoshiyama M, Tay FR, Torii Y, *et al*. Resin adhesion to carious dentin. Am J Dent 2003;16:47–52.
50. Arrais CA, Giannini M, Nakajima M, Tagami J. Effects of additional and extended acid etching on bonding to caries-affected dentine. Eur J Oral Sci 2004;112:458–464.
51. Doi J, Itota 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 2004;31:1023–1028.
52. Yazici AR, Akca T, Ozgünaltay G, Dayangaç B. Bond strength of a self-etching adhesive system to caries-affected dentin. Oper Dent 2004;29:176–181.
53. Yoshiyama M, Doi J, Nishitani Y, *et al*. Bonding ability of adhesive resins to caries-affected and caries-infected dentin. J Appl Oral Sci 2004;12:171–176.
54. Nakajima M, Kitasako Y, Okuda M, Foxton RM, Tagami J. Elemental distributions and microtensile bond strength of the adhesive interface to normal and caries-affected dentin. J Biomed Mater Res B Appl Biomater 2005;72:268–275.
55. Say EC, Nakajima M, Senawongse P, Soyman M, Ozer F, Tagami J. Bonding to sound vs caries-affected dentin using photo- and dual-cure adhesives. Oper Dent 2005;30:90–98.
56. Sonoda H, Banerjee A, Sherriff M, Tagami J, Watson TF. An in vitro investigation of microtensile bond strengths of two dentine adhesives to caries-affected dentine. J Dent 2005; 33:335–342.
57. Pereira PN, Nunes MF, Miguez PA, Swift EJ Jr. Bond strengths of a 1-step self-etching system to caries-affected and normal dentin. Oper Dent 2006;31:677–681.
58. Omar H, El-Badrawy W, El-Mowafy O, Atta O, Saleem B. Microtensile bond strength of resin composite bonded to caries-affected dentin with three adhesives. Oper Dent 2007; 32:24–30.
59. Erhardt MC, Rodrigues JA, Valentino TA, Ritter AV, Pimenta LA. In vitro micro TBS of one-bottle adhesive systems: sound versus artificially-created caries-affected dentin. J Biomed Mater Res B Appl Biomater 2008;6:181–187.
60. Xuan W, Hou BX, Lü YL. Bond strength of different adhesives to normal and caries-affected dentins. Chin Med J 2010;123:332–336.
61. Zanchi CH, Lund RG, Perrone LR, *et al*. Microtensile bond strength of two-step etch-and-rinse adhesive systems on sound and artificial caries-affected dentin. Am J Dent 2010;23:152–156.
62. Zanchi CH, D'Avila OP, Rodrigues-Junior SA, Burnett LH Jr, Demarco FF, Pinto MB. Effect of additional acid etching on bond strength and structural reliability of adhesive systems applied to caries-affected dentin. J Adhes Dent 2010;12:109–115.
63. Aggarwal V, Singla M, Miglani S. Effect of thermal and mechanical loading on marginal adaptation and microtensile bond strength of a self-etching adhesive with caries-affected dentin. J Conserv Dent 2011;14:52–56.
64. Mobarak EH, El-Badrawy WH. Microshear bond strength of self-etching adhesives to caries-affected dentin identified using the dye permeability test. J Adhes Dent 2012;14:245–250.
65. Alves FB, Lenzi TL, Reis A, Loguercio AD, Carvalho TS, Raggio DP. Bonding of simplified adhesive systems to caries-affected dentin of primary teeth. J Adhes Dent 2013;15:439–445.
66. Joves GJ, Inoue G, Nakashima S, Sadr A, Nikaido T, Tagami J. Mineral density, morphology and bond strength of natural versus artificial caries-affected dentin. Dent Mater J 2013; 32:138–143.
67. Erhardt MC, Lobo MM, Goulart M, *et al*. Microtensile bond strength of etch-and-rinse and self-etch adhesives to artificially created carious dentin. Gen Dent 2014;62:56–61.
68. Mobarak EH. Effect of chlorhexidine pretreatment on bond strength durability of caries-affected dentin over 2-year aging in artificial saliva and under simulated intrapulpal pressure. Oper Dent 2011;36:649–660.
69. Lenzi TL, Tedesco TK, Soares FZ, Loguercio AD, Rocha Rde O. Chlorhexidine does not increase immediate bond strength of etch-and-rinse adhesive to caries-affected dentin of primary and permanent teeth. Braz Dent J 2012;23:438–442.
70. Ekambaram M, Yiu CK, Matinlinna JP, King NM, Tay FR. Adjunctive application of chlorhexidine and ethanol-wet bonding on durability of bonds to sound and caries-affected dentine. J Dent 2014;42:709–719.

71. Pashley DH, Carvalho RM. Dentine permeability and dentine adhesion. J Dent 1997;5:355–372.
72. Watanabe I, Saimi Y, Nakabayashi N. Effect of smear layer on bonding to ground dentin. Relationship between grinding conditions and tensile bond strength. J Jpn Dent Mater 1994;13:101–108.
73. Toida T, Watanabe A, Nakabayashi N. Effect of smear layer on bonding to dentin prepared with bur. J Jpn Dent Mater 1995;14:109–116.
74. Spencer P, Wang Y, Walker MP, Swafford JR. Molecular structure of acid-etched dentin smear layers–in situ study. J Dent Res 2001;80:1802–1807.
75. Taniguchi G, Nakajima M, Hosaka K, *et al*. Improving the effect of NaOCl pretreatment on bonding to caries-affected dentin using self-etch adhesives. J Dent 2009;37: 769–775.
76. Spencer P, Wang Y, Walker MP, Swafford JR. Molecular structure of acid-etched dentin smear layers–in situ study. J Dent Res 2001;80:1802–1807.
77. Pashley DH, Ciucchi B, Sano H. Permeability of dentin to adhesive agents. Quintessence Int 1993;24:618–631.
78. Wang Y, Spencer P. Analysis of acid-treated dentin smear debris and smear layers using confocal Raman microspectroscopy. J Biomed Mater Res 2002;60:300–308.
79. Hashimoto M, Ohno H, Kaga M, Endo K, Sano H, Oguchi H. In vivo degradation of resin-dentin bonds in humans over 1 to 3 years. J Dent Res 2000;79:1385–1391.
80. Milia E, Santini A. Ultrastructural transmission electron microscopy (TEM) study of hybrid layers formed beneath a one-bottle adhesive system using the total-etch technique and a self-etching system. Quintessence Int 2003;34:447–452.
81. Tay FR, Pashley DH. Aggressiveness of contemporary self-etching systems. I. Depth of penetration beyond dentin smear layers. Dent Mater 2001;17:296–308.
82. Yoshida Y, Nagakane K, Fukuda R, *et al*. Comparative study on adhesive performance of adhesive monomers. J Dent Res 2004;83:454–458.
83. Yoshida Y, Van Meerbeek B, Nakayama Y, *et al*. Adhesion to and decalcification of hydroxyapatite by carboxylic acids. J Dent Res 2001;80:1565–1569.
84. Tay FR, Carvalho R, Sano H, Pashley DH. Effect of smear layers on the bonding of a self-etching primer to dentin. J Adhes Dent 2000;2:99–116.
85. Sano H, Yoshikawa T, Pereira PN, *et al*. Long-term durability of dentin bonds made with a self-etching primer, in vivo. J Dent Res 1999;78:906–911.
86. Okuda M, Pereira PN, Nakajima M, Tagami J. Relationship between nanoleakage and long-term durability of dentin bonds. Oper Dent 2001;26:482–490.
87. Nakabayashi N, Takarada K. Effect of HEMA on bonding to dentin. Dent Mater 1992;8:125–130.
88. Nakabayashi N, Watanabe A, Ikeda W. Intra-oral bonding of 4-META-MMA-TBBO resin to vital human dentin. Am J Dent 1995;8:37–42.
89. Milia E, Cumbo E, Cardoso RJ, Gallina G. Current dental adhesives systems. A narrative review. Curr Pharm Des 2012;18:5542–5552.
90. Ogawa K, Yamashita Y, Ichijo T, Fusayama T. The ultrastructure and hardness of the transparent layer of human caries dentin. J Dent Res 1983;62:7–10.
91. Marshall GW, Balooch M, Kinney JH, Marshall SJ. Atomic force microscopy of conditioning agents on dentin. J Biomed Mater Res 1995;29:1381–1387.
92. Pashley DH, Ciucchi B, Sano H. Permeability of dentin to adhesive agents. Quintessence Int 1993;24:618–631.
93. Attal JP, Asmussen G, Degrange M. Effects of surface treatment on the free surface of dentine. Dent Mater 1994;10: 259–264.
94. Jacsobsen T, Soderholm KJ. Some effects of water on dentin bonding. Dent Mater 1995;11:132–136.
95. Tay FR, Gwinnett JA, Wei SH. Micromorphological spectrum from overdrying to overwetting acid-conditioned dentin in water-free acetone-based, single-bottle primer⁄adhesives. Dent Mater 1996;12:236–244.
96. Mohsen NM, Craig RG, Filisko FE. The effects of moisture on the dielectric relaxation of urethane dimethacrylate polymer and composites. J Oral Rehabil 2001;28:376–392.
97. Sano H, Shono T, Takatsu T, Hosada H. Microporous dentin zone beneath resin-impregnated layer. Oper Dent 1994;19: 59–64.
98. Milia E, Lallai MR, Garcia-Godoy F. In vivo effect of a self-etching primer on dentin. Am J Dent 1999;4:167–171.
99. Kunawarote S, Nakajima M, Foxton RM, Tagami J. Pretreatment effect of mild acidic HOCl solution on adhesive to caries-affected dentin using self-etch adhesive. Eur J Oral Sci 2011;119:86–92.
100. Watanabe I, Nakabayashi N, Pashley DH. Bonding to ground dentin by a phenyl-P self-etching primer. J Dent Res 1994;73: 1212–1220.
101. Inoue S, Koshiro K, Yoshida Y, *et al*. Hydrolytic stability of self-etch adhesives bonded to dentin. J Dent Res 2005;84: 1160–1164.
102. Waidyasekera K, Nikaido T, Weerasinghe DS, Ichinose S, Tagami J. Reinforcement of dentin in self-etch adhesive technology: a new concept. J Dent 2009;37:604–609.
103. Ceballos L, Camejo DG, Victoria Fuentes M, *et al*. Microtensile bond strength of total-etch and self-etching adhesives to caries-affected dentine. J Dent 2003;31:469–477.
104. Hashimoto M, Ohno H, Kaga M, Endo K, Sano H, Oguchi H. In vivo degradation of resin-dentin bonds in humans over 1 to 3 years. J Dent Res 2000;79:1385–1391.
105. Sano H, Takatsu T, Ciucchi B, Horner JA, Matthews WG, Pashley DH. Nanoleakage: leakage within the hybrid layer. Oper Dent 1995;20:18–25.
106. Sano H, Yoshikawa T, Pereira PN, *et al*. Long-term durability of dentin bonds made with a self-etching primer, in vivo. J Dent Res 1999;78:906–911.
107. Hashimoto M, Ohno H, Sano H, *et al*. Micromorphological changes in resin-dentin bonds after 1 year of water storage. J Biomed Mater Res 2002;63:306–311.
108. Van Meerbeek B, De Munck J, Yoshida Y, *et al*. Buonocore memorial lecture. Adhesion to enamel and dentin: current status and future challenges. Oper Dent 2003;28:215–235.
109. Salz U, Zimmermann J, Zeuner F, Moszner N. Hydrolytic stability of self-etching adhesive systems. J Adhes Dent 2005;7:107–116.
110. Kwong SM, Tay FR, Yip HK, Kei IH, Pashley DH. An ultrastructural study of the application of dentine adhesives to acid-conditioned sclerotic dentine. J Dent 2000;28:515–528.
111. Santini A, Ivanivic V, Ibbetson R, Milia E. Influence of marginal bevels on microleakage around class V cavities bonded with seven self etching agents. Am J Dent 2004;17:257–261.
112. Inoue G, Nikaido T, Foxton RM, Tagami J. The acid-base resistant zone in three dentin bonding systems. Dent Mater J 2009;28:717–721.
113. Van Landuyt K, Snauwaert J, Peumans M, De Munck J, Lambrechts P, Van Meerbeek B. The role of HEMA in one-step self-etch adhesives. Dent Mater 2008;24:1412–1419.
114. Tay FR, Pashley DH. Water treeing – A potential mechanism for degradation of dentin adhesives. Am J Dent 2003;16: 6–12.

115. Itthagarun A, Tay FR, Pashley DH, Wefel JS, Garcia-Godoy F, Wei S. Single-step, self-etch adhesives behave as permeable membranes after polymerization. Part III. Evidence from fluid conductance and artificial caries inhibition. Am J Dent 2004;17:394–400.

116. Sauro S, Mannocci F, Toledano M, Osorio R, Thompson I, Watson TF. Influence of the hydrostatic pulpal pressure on droplets formation in current etch-and-rinse and self-etch adhesives: a video rate/TSM microscopy and fluid filtration study. Dent Mater 2009;25:1392–1402.

117. Hashimoto M, Ito S, Tay FR, *et al*. Fluid movement across the resin-dentin interface during and after bonding. J Dent Res 2004;83:843–848.

118. Grégoire G, Joniot S, Guignes P, Millas A. Dentin permeability: self-etching and one-bottle dentin bonding systems. J Prosthet Dent 2003;90:42–49.

119. Tay FR, Pashley DH, Hiraishi N, *et al*. Tubular occlusion prevents water-treeing and through-and-through fluid movement in a single-bottle, one-step self-etch adhesive model. J Dent 2005;84:891–896.

120. Carrilho MR, Carvalho RM, Tay FR, Yiu C, Pashley DH. Durability of resin-dentin bonds related to water and oil storage. Am J Dent 2005;18:315–319.

121. Santini A, Milia E, Miletic V. A review of SEM and TEM studies on the hybridisation of dentine. Microscopy: Science, Technology, Applications and Education 2010;1:256–268.

122. Reis AF, Giannini M, Pereira PN. Influence of water-storage time on the sorption and solubility behaviour of current adhesives and primer/adhesive mixtures. Oper Dent 2007;32: 53–59.

123. Vaidyanathan TK, Vaidyanathan J. Recent advances in the theory and mechanism of adhesive. Resin bonding to dentin: a critical review. J Biomed Mater Res Part B: Appl Biomater 2009;88:558–578.

124. Hebling J, Pashley DH, Tjäderhane L, Tay FR. Chlorhexidine arrests subclinical degradation of dentin hybrid layers in vivo. J Dent Res 2005;84:741–746.

125. Tersariol IL, Geraldeli S, Minciotti CL, *et al*. Cysteine cathepsins in human dentin-pulp complex. J Endod 2010;36:475–481.

126. Nishitani Y, Yoshiyama M, Wadgaonkar B, *et al*. Activation of gelatinolytic/collagenolytic activity in dentin by self-etching adhesives. Eur J Oral Sci 2006;114:160–166.

127. Lehmann N, Debret R, Romeas A, *et al*. Self-etching increases matrix metalloproteinase expression in the dentin-pulp complex. J Dent Res 2009;88:77–82.

128. Sulkala M, Larmas M, Sorsa T, Salo T, Tjaderhane L. The localization of matrix metalloproteinase-20 (MMP-20, enamelysin) in mature human teeth. J Dent Res 2002;81:603–607.

129. Carrilho MR, Geraldeli S, Tay F, *et al*. In vivo preservation of the hybrid layer by chlorhexidine. J Dent Res 2007;86: 529–533.

130. Visse R, Nagase H. Matrix metalloproteases and tissue inhibitors of metalloproteinases. Circ Res 2003;92:827–839.

131. Osorio R, Erhardt MCG, Pimenta LAF, Osorio F, Toledano M. EDTA treatment improves resin-dentin bonds resistance to degradation. J Dent Res 2005;85:736–740.

132. Pashley DH, Swift EJ Jr. Dentin bonding. J Esthet Restor Dent 2008;20:153–154.

133. Brackett MG, Tay FR, Brackett WW, *et al*. In vivo chlorhexidine stabilization of an acetone-based dentin adhesives. Oper Dent 2009;34:381–385.

134. Bedran-Russo AKB, Vidal CMP, Santos PHD, Castellan CS. Long-term effect of carbodiimide on dentin matrix and resin-dentin bonds. J Biomed Mater Res B Appl Biomater 2010;94:250–255.

135. Almahdy A, Koller G, Sauro S, *et al*. Effects of MMP inhibitors incorporated within dental adhesives. J Dent Res 2012;91:605–611.

136. Nishitani Y, Yoshiyama M, Donnelly AM, *et al*. Effects of resin hydrophilicity on dentin bond strength. J Dent Res 2006;85:1016–1021.

137. Pashley DH, Tay FR, Carvalho RM, *et al*. From dry bonding to water-wet bonding to ethanol-wet bonding. A review of the interactions between dentin matrix and solvated resins using a macromodel of the hybrid layer. Am J Dent 2007;20:7–20.

138. Gendron R, Grenier D, Sorsa T, Mayrand D. Inhibition of the activities of matrix metalloproteinases 2, 8, and 9 by chlorhexidine. Clin Diagn Lab Immunol 1999;6:437–439.

139. Erhardt MC, Osorio R, Toledano M. Dentin treatment with MMPs inhibitors does not alter bond strengths to caries-affected dentin. J Dent 2008;36:1068–1073.

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