Universal adhesives benefit from an extra hydrophobic adhesive layer when light-cured beforehand

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Clinical Relevance

Universal adhesives applied in self-etch mode on dentin may benefit from an extra hydrophobic adhesive layer when they are before light-cured.

Abstract

Objectives: To measure the 'immediate' and 6-month 'aged' micro-tensile bond strength (µTBS) of universal adhesives with or without an additional adhesive layer applied on a separately light-cured or non-light-cured universal adhesive.

Methods: Eighty human third molars were randomly assigned to 8 experimental groups. The universal adhesives Clearfil Universal Bond (Kuraray Noritake) and Single Bond Universal (3M Oral Care) were used in self-etch mode (following the manufacturer's directions), and either light-cured or not before application of an extra hydrophobic adhesive layer ('Clearfil SE Bond' bond, Kuraray Noritake). The two-step self-etch adhesives Clearfil SE Bond (Kuraray Noritake) and OptiBond XTR (Kerr) were used as references. After composite build-ups were bonded to mid-coronal occlusal dentin surfaces, the specimens were stored in water (37°C/24 hrs) and sectioned into micro-specimens (0.96±0.04 mm^2). Half of the specimens were immediately subjected to µTBS testing (1.0 mm/min), while the other half was stored in water (37°C) for 6 months prior to testing. Failure analysis was performed using stereomicroscopy and SEM. Data were analyzed with two-way repeated measures ANOVA, Tukey's and paired t tests ($p=0.05$).

Results: The immediate µTBS was alike for the universal adhesives when applied following the different application strategies ($p>0.05$). Application of an extra hydrophobic adhesive layer improved the aged µTBS of the universal adhesives, which became statistically significant when the universal adhesives were beforehand light-cured ($p<0.05$). The reference adhesives Clearfil SE Bond and OptiBond XTR exhibited a significantly higher immediate and aged µTBS to dentin than the universal adhesives ($p<0.05$).

Conclusion: The bond durability of universal adhesives, applied in self-etch mode, was found to benefit from the application of an extra hydrophobic adhesive layer when the universal adhesives were before light-cured.

Keywords: Aging, Dentin, Micro-tensile bond strength, Self-etch adhesives

Introduction

Current adhesive technology tends to simplify bonding procedures by reducing application steps, shortening clinical application time and decreasing technique sensitivity.[42] Recently, a new generation of adhesives has been introduced. These adhesives are termed as 'universal' or 'multi-mode', as they can be applied either in an etch-and-rinse or self-etch mode;[10,23,25] the third application option involves their application on phosphoric-acid pre-etched tooth enamel (and unetched dentin) following a so-called selective enamel-etching approach to achieve better bond durability at enamel,[10,23,25] while maintaining the self-etch approach on dentin. The latter adhesive protocol preserves the potential to achieve additional primary chemical bonding with the remaining (carbonated) apatite crystallites at dentin (and enamel).[42,50] The manufacturers claim that one adhesive solution can be used for either adhesive strategy without compromising bonding effectiveness, by which these universal adhesives do replace the previous generation of simplified non-universal adhesives that needed to be applied either following an etch-and-rinse or a self-etch approach. The optional etch-and-rinse or self-etch application mode of universal adhesives has been made possible through the inclusion of hydrophilic functional monomers along with increased solvent amount, so to make these adhesives compatible with both a non-pre-etched (self-etch mode) or pre-etched (etch-and-rinse mode) wet dentin substrate.[20,49]

However, the adapted adhesive formulations of universal adhesives have been documented to lead to more residual solvent entrapment within the adhesive layer.[49] Such residual solvents may hinder the formation of a highly cross-linked polymer, hereby decreasing the polymerization-conversion degree within the adhesive layer, which on its turn may affect the adhesive-dentin bond strength and increase the permeability of the adhesive layer after polymerization.[15,47] Consequently, these effects may compromise the final structure of the polymer and its mechanical properties, making it more vulnerable to accelerated bond degradation.[15,47,49]

It has been advocated that the short- and long-term bonding effectiveness of one-step self-etch adhesives can be improved by the placement of an additional hydrophobic resin coat.[1,13,24]

Therefore, one method to overcome such deficiencies of universal adhesives involves the application of an additional layer of a hydrophobic resin to coat the adhesive. This extra resin coat will increase the thickness and uniformity of the adhesive layer with the expectation to reduce fluid flow across the adhesive interface[1,13,24] However, with a thicker adhesive layer it may be more difficult to volatilize the solvent before light-curing and so negatively affect bond strength.[56] The solution to this problem could be to light-cure the adhesive layer separately prior to the application of the extra hydrophobic resin. Only a few laboratory studies have reported that placement of a hydrophobic resin coating over the polymerized universal adhesives indeed improved the micro-tensile dentin bond strength (μTBS) of universal adhesives when used in self-etch mode.[20,29] Nevertheless, the effect of the application of an additional hydrophobic adhesive layer on a separately light-cured or non-light-cured universal adhesive has not been tested so far.

The aim of this study was therefore to evaluate the immediate (24 hrs) and aged (6 months) μTBS of two universal adhesives used in self-etch mode on dentin with or without an additional hydrophobic adhesive layer applied on the separately light-cured or non-light-cured universal adhesive. The hypotheses tested were (1) that the use of an additional hydrophobic adhesive layer applied on a non-light-cured universal adhesive would not improve both the immediate (1a) and aged (1b) bond strength to dentin, and (2) that the use of an additional hydrophobic adhesive layer applied on a separately light-cured universal adhesive would not improve both the immediate (2a) and aged (2b) bond strength to dentin.

Materials and Methods

Tooth selection and preparation

Eighty extracted caries-free human third molars were used following ethical approval (ref no: 06.01.2016/4). In this study, the teeth were disinfected in 0.5% chloramine, stored in distilled water and used within three months after extraction. The mid-coronal dentin surface was exposed in all teeth after sectioning the occlusal enamel using a water-cooled diamond saw (Minitom, Struers, Ballerup, Denmark). The dentin surfaces were controlled for absence of enamel and/or pulp tissue using a stereo-microscope (S4E, Leica Microsystems, Wetzlar, Germany). The exposed dentin surfaces were further ground with wet #320-grit silicon carbide paper (MD Fuga, Struers, Ballerup, Denmark) for 60 s to standardize the preparation of smear layers (Fig. 1).

Experimental design and specimen preparation

The teeth were randomly assigned into eight groups (n=10) according to the different adhesive strategies of the respective adhesive (Table 1 and Table 2). The two universal adhesives were solely applied in self-etch mode: Clearfil Universal Bond (Kuraray Noritake, Tokyo, Japan) and Single Bond Universal (3M Oral Care, Seefeld, Germany; also marketed as Scotchbond Universal in other countries). As references, the two-step self-etch adhesives Clearfil SE Bond (Kuraray Noritake) and Optibond XTR (Kerr, Orange, CA, USA) were used. The bonding agent of Clearfil SE Bond ('Clearfil SE Bond' bond, Kuraray Noritake) was also used as extra hydrophobic resin. In the experimental groups that additionally received an extra hydrophobic resin, each universal adhesive was applied according to the manufacturer's directions with the adhesive either separately light-cured or not prior to the application of an additional coat of the hydrophobic resin layer ('Clearfil SE Bond' bond, Kuraray Noritake). When the hydrophobic resin layer was additionally applied, it was extra light-cured for 10 s (Table 2).

After adhesive procedures, composite build-ups were prepared using a nanofilled composite (Filtek Ultimate, 3M Oral Care; marketed as Filtek Supreme XTE in other countries) in increments of 2 mm each to a height of 5-6 mm. Each increment was light-cured for 20 s using an LED light-curing unit (Valo, Ultradent, St. Louis, MO, USA) with a light output of 1000 mW/cm^2. After storage in distilled water for 24 hrs at 37°C, the specimens were sectioned longitudinally across the bonded interface in mesio-distal and buccal-lingual directions with a slow-speed diamond saw (Minitom, Struers) to obtain composite-adhesive-dentin sticks with a cross-sectional area of 0.96±0.04 mm^2, as was measured using a digital caliper (Digimatic Caliper, Mitutoyo, Tokyo, Japan). Half of the sticks from the central and peripheral region of each tooth were used to measure the 'immediate' (24 hrs) μTBS; the other half were stored in distilled water for 6 months at 37°C to determine the 'aged' (6-month) μTBS following the same test protocol.

Micro-tensile bond strength testing

The composite-dentin bonded sticks were attached to a modified Geraldeli's BIOMAT micro-tensile bond-strength testing jig with cyanoacrylate glue (Zapit, Dental Ventures of America, Corona, CA, USA) and stressed at a crosshead speed of 1 mm/min until failure in a LRX testing device (LR 5K Lloyd, Lloyd, Hampshire, UK) using a load cell of 100 N. After measuring the exact dimensions of each fractured stick with the digital caliper (Digimatic Caliper, Mitutoyo), the μTBS was calculated in MPa, as derived from dividing the imposed force (in N) at the time of fracture by the bond area (in mm^2). When specimens failed before actual testing (pre-testing failures or 'ptf'), they were included as 0 MPa in the calculation of the mean μTBS.

For each experimental group, the mean of μTBS values of the sticks originating from the same tooth were calculated and the mean bond strength was used as one unit for statistical analysis. Data were submitted to two-way repeated measures ANOVA. The 'adhesive strategy' (universal adhesive without hydrophobic adhesive layer, light-cured or non-light-cured universal adhesive with extra hydrophobic adhesive layer, two-step self-etch adhesive) and 'storage time' were the two main factors. The Tukey's post-hoc multiple comparison test was used for pairwise comparisons. To compare the means regarding storage time (24 hrs or 6 months) paired samples t test was performed. A significance level of 0.05 was used for all tests.

Failure analysis

The failure mode analysis was performed under a stereomicroscope at ×80 magnification. The failure mode was classified as 'adhesive failure', 'mixed failure', 'cohesive failure in composite' or 'cohesive failure in dentin', the latter when more than one of the beforementioned fracture modes occurred at one fractured surface. Four representative specimens of each experimental group were selected. Each specimen was fixed onto an aluminum SEM sample holder with carbon glue and observed using scanning electron microscopy (SEM; Quanta Feg 250, FEI, Eindhoven, The Netherlands).

Results

Micro-tensile bond strength

Two-way ANOVA disclosed statistical differences for the main factors 'adhesive strategy' ($p=0.000$) and 'storage time' ($p=0.000$), and for their interaction ($p=0.025$) (Table 3). The overall mean μTBS of all experimental groups, including the standard deviations, the number of pre-testing failures (ptf's) and specimens (n) are detailed in Table 4 and graphically presented in Figure 2. The results of multiple comparisons statistical analysis are also shown in Table 4.

Regarding immediate bond strength, the use of an extra hydrophobic resin coating with beforehand light-curing or without light-curing of the universal adhesive did not significantly influence the mean μTBS of the universal adhesives ($p>0.05$). Both universal adhesives tested resulted in lower mean μTBSs than the reference two-step self-etch adhesives ($p<0.05$). Significant differences in mean μTBS were neither detected between the reference two-step self-etch adhesives ($p>0.05$).

Regarding aged bond strength, application of an extra hydrophobic resin coating increased the aged μTBS of the universal adhesives, but this became only statistically significant when the universal adhesives were beforehand light-cured ($p<0.05$). The two universal adhesives tested resulted in a lower aged μTBS than the reference two-step self-etch adhesives ($p<0.05$). No significant differences were detected between the reference two-step self-etch adhesives ($p>0.05$).

Storage time significantly reduced the mean µTBS of all test groups ($p<0.05$), except for light-cured Clearfil Universal Bond with hydrophobic adhesive layer, which scored similarly for both storage times ($p>0.05$).

Failure analysis

Pre-testing failures were recorded for all universal adhesive experimental groups, although they were relatively low in number (5.5%). No pre-testing failures were recorded for the two reference two-step self-etch adhesives. All pre-testing failures occurred during sectioning of the sticks with the diamond saw and these failures occurred adhesively. The results of the light-microscopy failure analysis are graphically presented in Figure 3. Most specimens failed adhesively and the least specimen numbers failed cohesively in dentin, irrespective of adhesive, experimental condition or storage time. Some representative SEM photomicrographs are illustrated in Figure 4.

Discussion

In recent years, the application of an extra hydrophobic resin layer has been recommended to improve the adhesion of one-step (self-etch) and universal adhesives to dentin.[1,13,20,23,24,29] In this study, the two universal adhesives Clearfil Universal Bond (Kuraray Noritake) and Single Bond Universal (3M Oral Care) were investigated with the adhesive separately light-cured or not, prior to the extra application of 'Clearfil SE Bond' bond (Kuraray Noritake). Although higher µTBSs of the universal adhesives to dentin were achieved upon use of the extra hydrophobic layer, only when the universal adhesive was separately light-cured before the application of 'Clearfil SE Bond' bond (Kuraray Noritake), the dentin bond strength significantly improved. When the universal adhesive was not beforehand light-cured and thus used as a kind of primer prior to the extra hydrophobic resin layer, no significant increase in bond strength was recorded. Therefore, the hypothesis that the use of an additional hydrophobic adhesive layer applied on a non-light-cured universal adhesive would not improve both the immediate (1a) and aged (1b) bond strength on dentin, was accepted. In previous studies conducted elsewhere, however, significantly higher dentin bond strengths were obtained using universal adhesives (All-Bond Universal, Bisco, Schaumburg, IL, USA; Single Bond Universal, 3M Oral Care) applied in self-etch mode with being separately light-cured prior to the application of an extra hydrophobic resin coating.[20,29] In these studies, the application of the universal adhesives was followed by one coat of the hydrophobic resin Heliobond (Ivoclar Vivadent, Schaan, Liechtenstein). The authors attributed the improved bonding effectiveness to the improved mechanical interfacial properties by decreasing the concentration of retained solvents and unreacted monomers in the adhesive layer,[6,20,29] and also thanks to the increased thickness of the adhesive layer.[24,29]

In the current study, a beneficial effect of the extra hydrophobic resin layer was obtained only when the universal adhesives were separately light-cured before the extra layer of 'Clearfil SE Bond' bond (Kuraray Noritake) was applied and this solely for the aged dentin bond strength. Consequently, the hypothesis that the use of an additional hydrophobic adhesive layer applied on a separately light-cured universal adhesive would not improve the immediate (2a) and aged (2b) bonding performance onto dentin, was accepted regarding immediate bond strength, but failed to be accepted regarding aged bond strength. Simplified one-step adhesives that combine the primer function with that of the adhesive resin in multi-step adhesives, are generally more hydrophilic and contain more solvent. As a result, these adhesives have been documented to behave as semi-permeable membranes[5,27,34,35] and do commonly reach less high polymerization-conversion rates in part due to remaining solvent.[3,22] In this way, these adhesives are more prone to hydrolytic degradation processes, as the resultant adhesive interface is less hydrophobic and thus blocks less watersorption through osmosis from the underlying dentin[5,33,39,40] or from the external environment.[24,29,33] Somewhat unexpected in this study was that the extra application of the hydrophobic resin layer on top of the universal adhesive that was solely employed as primer and not separately light-cured, appeared insufficient to result in a more aging-resistant adhesive interface. Indeed, the highest bond-strength data after 6 months of water storage were observed in the experimental group in which the extra hydrophobic resin layer was applied on top of beforehand light-cured universal adhesives. Previous laboratory research revealed bonding performance improving with thickness of the adhesive layer; increased bond strengths were achieved by applying multiple adhesive coats that each were separately light-cured.[1,11] Universal adhesives commonly have a rather thin film thickness (<10 μm), in particular as more solvent evaporation by air-blowing/thinning may be indicated because of their higher solvent content.[2] Separately light-curing the universal adhesives used in self-etch mode before application of the extra layer of 'Clearfil SE Bond' (Kuraray Noritake) may have thickened the adhesive layer and improved the aging resistance.

Both the universal adhesives applied under the different experimental conditions and the two two-step self-etch reference adhesives presented with significantly lower bond strengths upon 6-month ageing than at 24 hrs (immediate μTBS). The only exception is the universal adhesive Clearfil Universal Bond (Kuraray Noritake) when it was separately light-cured and covered by the extra hydrophobic adhesive layer (CUB_{LC}+C-SE_{bond}); then, not statistically significantly different bond strengths were recorded at the two time points. Also, in other long-term bonding effectiveness studies, Clearfil Universal Bond (Kuraray Noritake)[4,55] and Single Bond Universal (3M Oral Care),[4,17,26,29,43] when applied in self-etch mode, were shown incapable of producing similar μTBS to dentin when the adhesives were applied without an extra hydrophobic adhesive layer irrespective of specimen aging. Some studies, however, reported otherwise; the application of Clearfil Universal Bond (Kuraray Noritake)[8] and Single Bond Universal (3M Oral Care)[19,36,44,46,55] in self-etch mode without an hydrophobic adhesive layer resulted in similar dentin bonding effectiveness initially as upon six-month or one-year aging. In one study that also investigated the influence of hydrophobic resin coating on bonding effectiveness, the authors showed that Single Bond Universal (3M Oral Care) applied in self-etch mode did not result in a significant reduction after 6-month aging when a hydrophobic resin coating was applied; significantly reduced 6-month bond strength was recorded when no extra hydrophobic resin coating was applied.[29] However, in the same study, the aged 6-month μTBS was not statistically different from that recorded at 24 hrs for All-Bond Universal (Bisco) with or without an extra hydrophobic coating applied on a separately light-cured universal adhesive used in self-etch mode. The authors concluded that aging stability was material dependent.[29] Recently some clinical studies have indicated a good performance for the universal adhesive All-Bond Universal (Bisco) as well as for the two-step self-etch adhesive Optibond XTR (Kerr), in Class II composite restorations after three years of clinical service.[38]

Both the universal adhesives Clearfil Universal Bond (Kuraray Noritake) and Single Bond Universal (3M Oral Care) contain the monofunctional monomer HEMA as hydrophilic monomer (Table 1). HEMA is often added to adhesive formulations (1) to promote wetting of the hydrophilic dentin surface, (2) to facilitate resin infiltration thanks to its hydrophylicity and small size, this in particular for etch-and-rinse adhesives/modes, and (3) because of its capacity to act as solvent for other methacrylate monomers, hereby avoiding phase separation.[21,31,39,41] Disadvantageously, the higher hydrophilicity of HEMA and HEMA's lower polymerization potential as monomethacrylate monomer cause HEMA-rich adhesives to absorp more water than HEMA-free/poor adhesives.[16,31] Long-term water storage was shown to reduce the tensile strength of adhesives in a degree relative to their hydrophilicity.[7,9,16,30,48] Interfacial biodegradation is also induced by activated endogenic matrix metalloproteinases and cathepsins, a process also requiring an aqueous environment.[12,14,28,36] In this respect, a water-poor and highly hydrophobic interface that minimizes watersorption is desirable.

Both the immediate 24 hrs and aged 6 months bonding performance to dentin of the two universal adhesives Clearfil Universal Bond (Kuraray Noritake) and (Single Bond Universal, 3M Oral Care) used in this study in self-etch mode did not significantly differ from each other at the two measurement time points. Both adhesives include 10-MDP (10-methacryloyloxydecyl dihydrogen phosphate) as acidic functional monomer, which besides creating surface micro-retention also primary chemically interacts with calcium in hydroxyapatite.[52,53] Single Bond Universal (3M Oral Care) also contains a polyalkenoic-acid co-polymer, which can also bond chemically to hydroxyapatite,[4,51] although it is not clear if the co-polymer is added in a sufficiently high percentage to be chemically interactive. In this respect, the polyalkenoic-acid co-polymer has also been reported to potentially compete with the 10-MDP functional monomer for calcium-bonding sites in hydroxyapatite, as well it may inhibit monomer approximation during polymerization due to its high molecular weight.[20,44] In previous studies, Single Bond Universal (3M Oral Care) was demonstrated to outperform Clearfil Universal Bond (Kuraray Noritake) in terms of bond strength to dentin when both were used in self-etch mode,[4,37] hereby differing from our study results. Otherwise, the mean dentin μTBS of Single Bond Universal (3M Oral Care) obtained in this study is in agreement with previous studies conducted elsewhere, in which the bond strength values range from 27.6 to 59.9 MPa depending on the kind of experiment.[4,8,17-20,26,29,32,36,37,43,44,46,55]

Favorable findings were recorded for the reference two-step self-etch adhesives Clearfil SE Bond (Kuraray Noritake) and Optibond XTR (Kerr); the universal adhesives investigated significantly underperformed the two-step self-etch adhesives. Noteworthy is the significantly higher bonding performance of the reference adhesives for both the immediate and aged μTBS as compared to the universal adhesives applied in all the different adhesive modes. Likewise, no pre-testing failures were recorded for the reference adhesives in contrast with all experimental groups of the universal adhesives, while the number of pre-testing failures still can be considered relatively low. The bond strength results in other studies that investigated the same universal and reference adhesives, tend to agree with our findings.[8,18,19,26,37] The stable bonding effectiveness of the mild two-step self-etch adhesive Clearfil SE Bond (Kuraray Noritake) is believed to be related to the 10-MDP functional monomer, probably along with other properties of primary importance with regard to bonding effectiveness and bond durability, such as efficient polymerization and the application of a separate solvent-low and hydrophobic bonding agent that does not contain solvent but 10-MDP and HEMA.[50-53] In contrast to Clearfil SE Bond (Kuraray Noritake), the two-step self-etch adhesive Optibond XTR contains GPDM (glycerol phosphate dimethacrylate). The new self-etch adhesive is claimed to feature an enhanced etching ability of the primer. Such ability results from the rapid evaporation of acetone that concentrates water and GPDM monomers, thus lowering the pH from the initial value of 2.4 to 1.6.[45] The interfacial chemical interaction of GPDM with hydroxyapatite and dentin has recently been characterized,[54] despite the monomer has been ultilized in adhesives since long. Data suggested a weak bond between GPDM and hydroxyapatite and unstable calcium salts, although GPDM adsorbed to hydroxyapatite.[46,54]

In conclusion, the results indicate that the bond durability of the two universal adhesives investigated, applied in self-etch mode, benefited from the application of an extra hydrophobic adhesive layer, but solely when the universal adhesive was beforehand separately light-cured. Nevertheless, the universal adhesives investigated revealed lower immediate and aged micro-tensile bond strengths when applied following the different adhesive strategies than the two reference two-step self-etch adhesives. Self-evidently, long-term clinical randomized clinical trials are needed to validate the laboratory bonding performances.

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Table 1. List of the adhesives investigated, along with their chemical composition and application procedure.

Adhesive (manufacturer)	**Composition***	**Application procedure**
Clearfil Universal Bond (Kuraray Noritake, Tokyo, Japan) Lot no: 3D0006	10-MDP, Bis-GMA, hydrophilic aliphatic DM, HEMA, colloidal silica, camphorquinone, silane, accelerators, initiators, ethanol, water	1. Apply the adhesive to the entire cavity wall with the applicator brush and rub it in for 10 s; 2. Dry the cavity wall sufficiently by blowing mild air for more than 5 s until the adhesive shows no movement; use a vacuum aspirator to prevent the adhesive from scattering; 3. Light-cure for 10 s.
Single Bond Universal** (3M Oral Care, Seefeld, Germany) Lot no: 609973	10-MDP, DM, HEMA, methacrylate-modified polyalkenoic acid copolymer, filler, initiators, silane, ethanol, water	1. Apply the adhesive to the entire preparation with a microbrush and rub it in for 20 s; 2. Direct a gentle stream of air over the liquid for about 5 s until it no longer moves and the solvent is evaporated completely; 3. Light-cure for 10 s.
Clearfil SE Bond (Kuraray Noritake) Lot no: 000156	*Primer*: 10-MDP, hydrophilic DM, HEMA, camphorquinone, water *Bond*: 10-MDP, Bis-GMA, hydrophobic DM, HEMA, camphorquinone, N,N-diethanol p-toluidine bond, colloidal silica	1. Apply *Primer* to tooth surface and leave in place for 20 s; 2. Dry with air stream to evaporate the volatile ingredients; 3. Apply *Bond* to the tooth surface and then create a uniform film using a gentle air stream; 4. Light-cure for 10 s.
Optibond XTR (Kerr, Orange, CA, USA) Lot no: 5351340	*Primer*: GPDM, hydrophilic co-monomers, ethanol, acetone, water *Bond*: Resin monomers, HEMA, inorganic fillers, ethanol	1. Scrub *Primer* with a brushing motion for 20 s; 2. Air thin for 5 s; 3. Apply *Bond* for 15 s; 4. Light-cure for 10 s.

*Composition as provided by the respective manufacturers: Bis-GMA, bisphenol A-glycidyl methacrylate; DM, dimethacrylate; GPDM, glycerol phosphate dimethacrylate; HEMA, hydroxyethylmethacrylate; 10-MDP, 10-methacryloyloxydecyl dihydrogen phosphate; **Commercialized as Scotchbond Universal in other parts of the world.

Table 2. Overview of the experimental groups.

Adhesive	Adhesive strategy / Abbreviation	Experimental group code
Universal adhesive used in SELF-ETCH mode	Light-cured **Clearfil Universal Bond** without hydrophobic adhesive layer	CUB
	Non-light-cured Clearfil Universal Bond with light-cured hydrophobic adhesive layer	CUB_{nonLC}+C-SE_{bond}
	Light-cured Clearfil Universal Bond with light-cured hydrophobic adhesive layer	CUB_{LC}+C-SE_{bond}
	Light-cured **Single Bond Universal** without hydrophobic adhesive layer	SBU
	Non-light-cured Single Bond Universal with light-cured hydrophobic adhesive layer	SBU_{nonLC}+C-SE_{bond}
	Light-cured Single Bond Universal with light-cured hydrophobic adhesive layer	SBU_{LC}+C-SE_{bond}
Two-step self-etch adhesive (reference)	**Clearfil SE Bond**	C-SE
	OptiBond XTR	O-XTR

Table 3. Two-way ANOVA for the micro-tensile bond strength.

Source	Sum of squares	df	Mean square	F	p
Adhesive strategy	5944.706	7	849.244	173.112	0.000*
Storage time	1320.489	1	1320.489	107.843	0.000*
Interaction	211.566	7	30.224	2.468	0.025*

*Statistically significant differences (p<0.05)

Table 4. The mean micro-tensile bond strength (μTBS in MPa±SD) of the different experimental groups.

Experimental groups	Immediate μTBS (24 hrs)		Aged μTBS (6 months)	
	MPa±SD	ptf/n	MPa±SD	ptf/n
CUB	32.42±3.45^{aA}	5/45	23.72±2.87^{cB}	5/45
CUB$_{nonLC}$+C-SE$_{bond}$	33.76±3.64^{aA}	3/43	26.59±2.73^{cdB}	4/44
CUB$_{LC}$+C-SE$_{bond}$	34.48±2.78^{aA}	2/43	31.03±3.32^{efA}	3/43
SBU	34.03±3.69^{aA}	3/43	24.81±2.83^{cdB}	4/44
SBU$_{nonLC}$+C-SE$_{bond}$	34.46±3.30^{aA}	3/44	28.25±3.03^{deB}	3/43
SBU$_{LC}$+C-SE$_{bond}$	36.38±3.35^{aA}	2/44	32.26±3.28^{fB}	3/43
C-SE	47.08±1.56^{bA}	0/50	42.62±1.40^{gB}	0/50
O-XTR	45.38±2.77^{bA}	0/50	42.75±1.36^{gB}	0/50

SD = standard deviation; ptf = pre-testing failures (included as 0 MPa); n = total number of specimens (n differs among the experimental groups because teeth differ in size and can reveal different numbers of sticks; 10 teeth per experimental group); same small/capital letter indicates no statistical difference in the columns and rows, respectively.

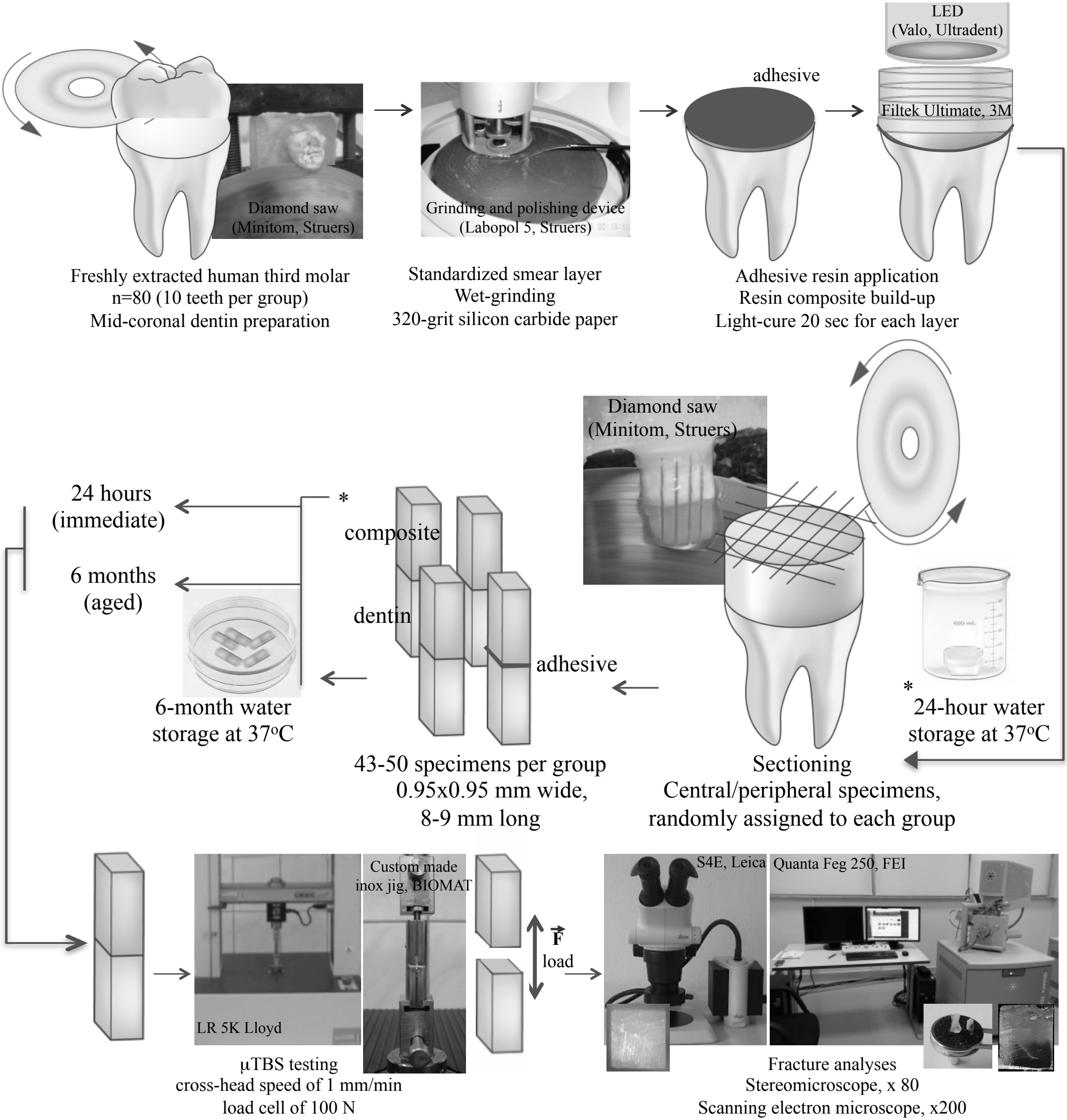
Captions to figures

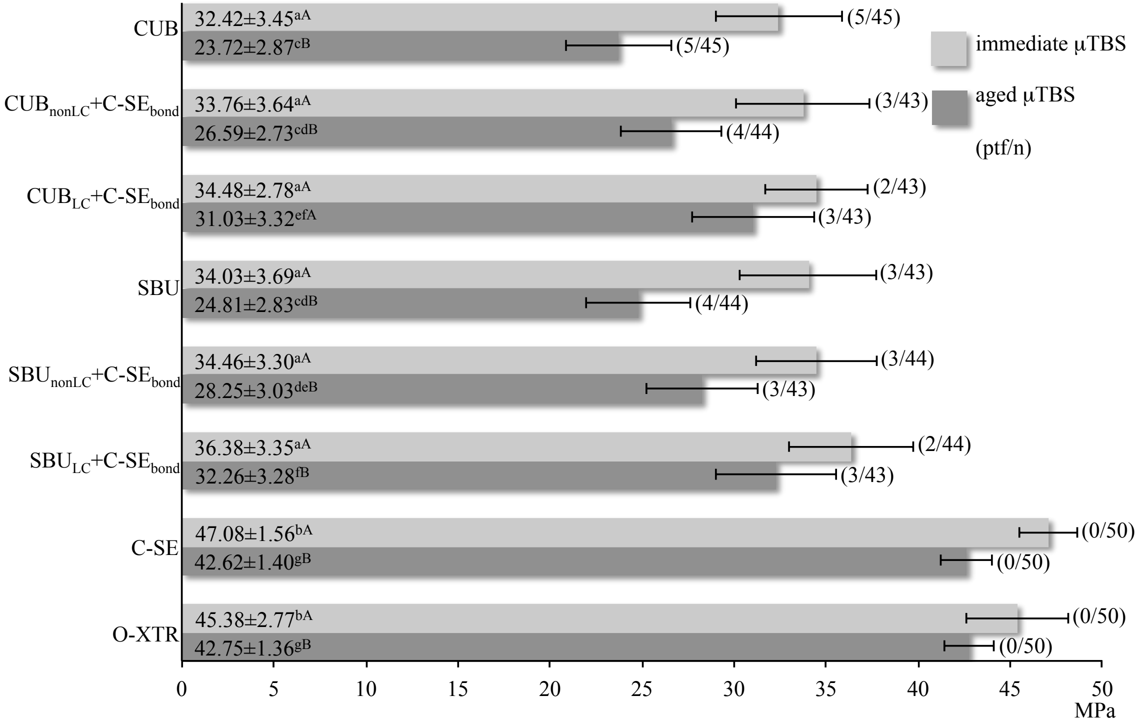
Figure 1. Schematic illustrating the experimental study design.

Figure 2. Micro-tensile bond strength (μTBS in MPa±SD) of the adhesives to dentin following the different experimental groups. Means and standard deviations are represented inside the bars. Means with the same superscript are not significantly different from each other. The number of pre-testing failures per total number of specimens (ptf/n) is also noted between the parenthesis after each bar.

Figure 3. Graphical presentation of the incidence (%) of failure modes observed using light-microscopy.

Figure 4. Overview SEM photomicrographs of the fracture surfaces (dentin and composite counterpart) of specimens produced by non-light-cured/light-cured Clearfil Universal Bond (Kuraray Noritake) and Single Bond Universal (3M Oral Care) followed by the application of an extra hydrophobic adhesive layer; adhesive and mixed failure patterns were revealed. (a) **CUB_{nonLC}+C-SE_{bond} immediate:** Large part of the total surface area of the specimen failed interfacially (adhesive failure), which was typically recognized by scratch marks remaining from the laboratory prepared dentin smear layer (arrows). Part of the adhesive resin chipped off during testing. (b) **CUB_{LC}+C-SE_{bond} aged**: Cohesive failure of the adhesive resin, cohesive failure in the composite resin and interfacial failure (arrows) typically occurred in this specimen (mixed failure). Many small porosities can be observed in the adhesive resin itself (block arrows, composite counterpart). (c) **SBU_{nonLC}+C-SE_{bond} immediate:** A small part of the specimen failed near the interface and a large part failed within the adhesive resin (recorded as adhesive failure). (d) **SBU_{LC}+C-SE_{bond} aged:** The specimen failed at the interface (adhesive failure). Smooth appearance of the adhesive resin left on the composite side can be observed.


LED
(Valo, Ultradent)
adhesive
Filtek Ultimate, 3M
Diamond saw
(Minitom, Struers)
Grinding and polishing device
(Labopol 5, Struers)
Freshly extracted human third molar
n=80 (10 teeth per group)
Mid-coronal dentin preparation
Standardized smear layer
Wet-grinding
320-grit silicon carbide paper
Adhesive resin application
Resin composite build-up
Light-cure 20 sec for each layer
Diamond saw
(Minitom, Struers)
24 hours
(immediate)
6 months
(aged)
*
composite
dentin
adhesive
6-month water
storage at 37°C
*
24-hour water
storage at 37°C
43-50 specimens per group
0.95x0.95 mm wide,
8-9 mm long
Sectioning
Central/peripheral specimens,
randomly assigned to each group
Custom made
inox jig, BIOMAT
LR 5K Lloyd
F
load
S4E, Leica
Quanta Feg 250, FEI
μTBS testing
cross-head speed of 1 mm/min
load cell of 100 N
Fracture analyses
Stereomicroscope, x 80
Scanning electron microscope, x200


CUB
32.42±3.45^aA
(5/45)
23.72±2.87^cB
(5/45)
CUB_{nonLC}+C-SE_{bond}
33.76±3.64^aA
(3/43)
26.59±2.73^cdB
(4/44)
CUB_{LC}+C-SE_{bond}
34.48±2.78^aA
(2/43)
31.03±3.32^efA
(3/43)
SBU
34.03±3.69^aA
(3/43)
24.81±2.83^cdB
(4/44)
SBU_{nonLC}+C-SE_{bond}
34.46±3.30^aA
(3/44)
28.25±3.03^deB
(3/43)
SBU_{LC}+C-SE_{bond}
36.38±3.35^aA
(2/44)
32.26±3.28^fB
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C-SE
47.08±1.56^bA
(0/50)
42.62±1.40^gB
(0/50)
O-XTR
45.38±2.77^bA
(0/50)
42.75±1.36^gB
(0/50)
0
5
10
15
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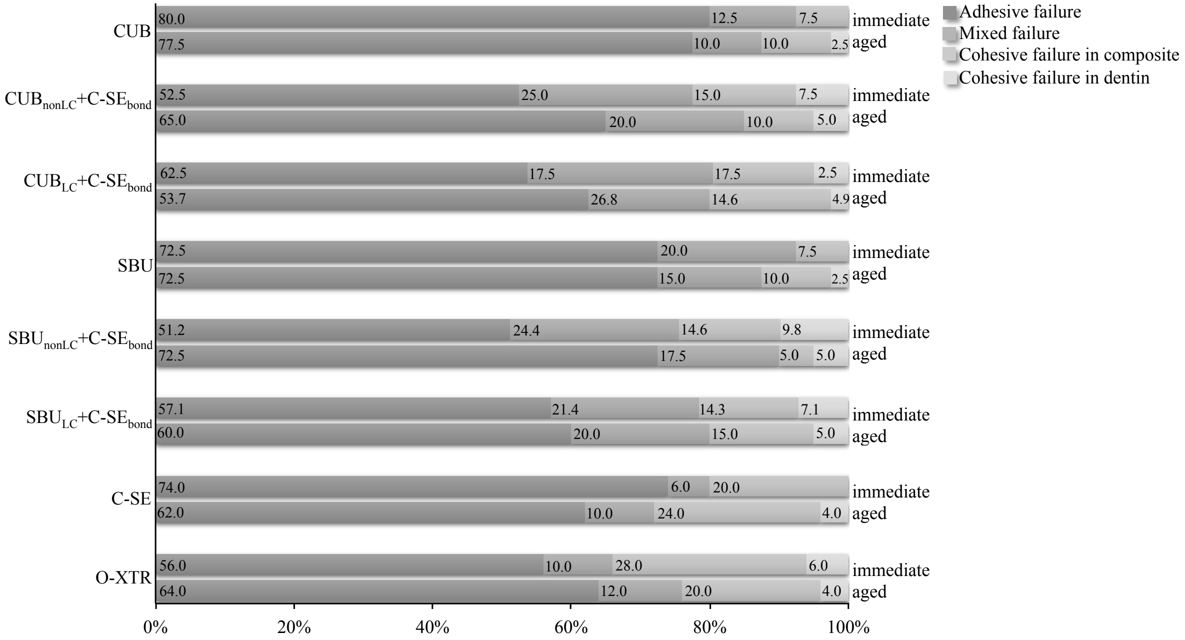
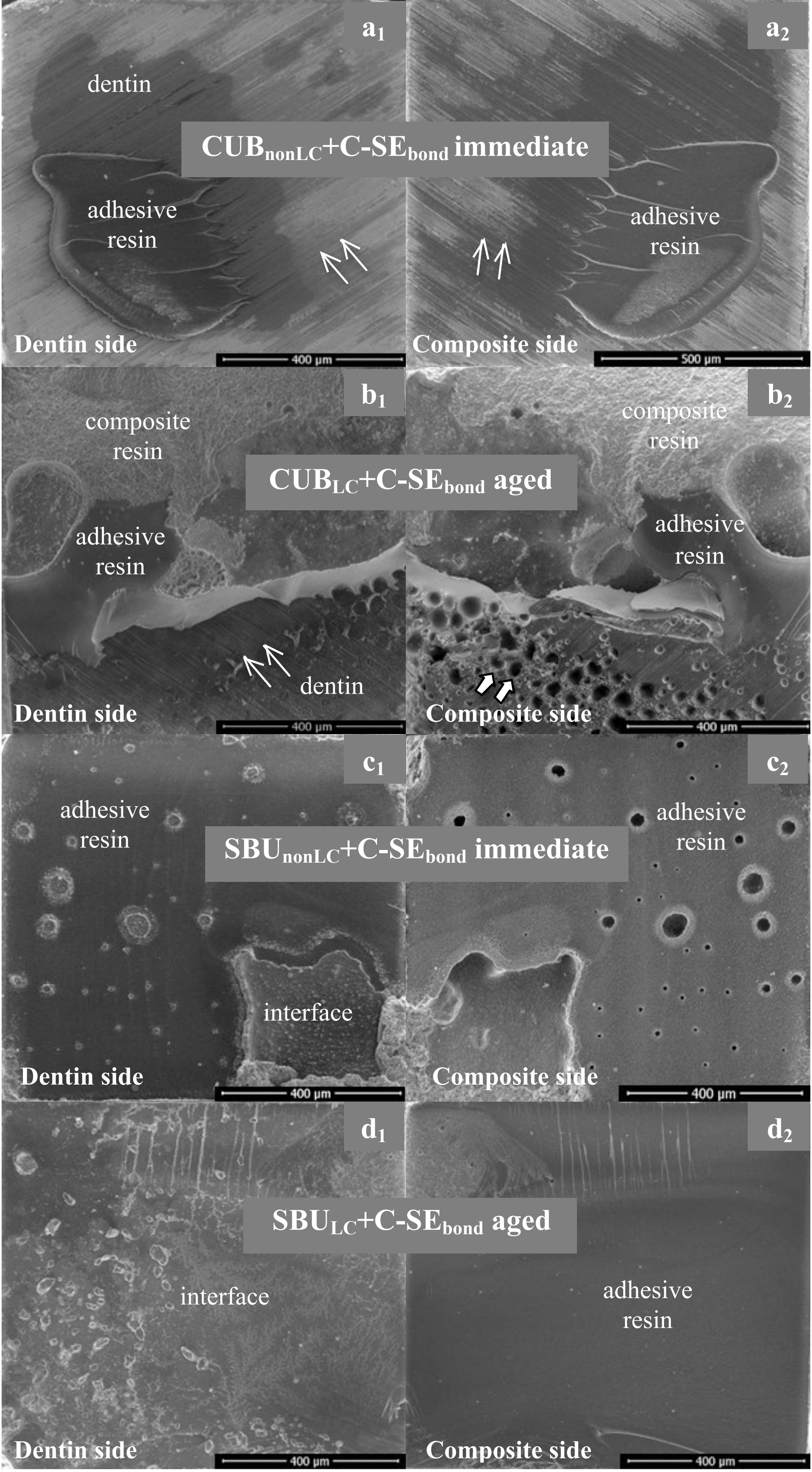
35
40
45
50
MPa
immediate μTBS
aged μTBS
(ptf/n)


Adhesive failure
Mixed failure
Cohesive failure in composite
Cohesive failure in dentin
CUB
80.0 12.5 7.5 immediate
77.5 10.0 10.0 2.5 aged
CUB$_{nonLC}$+C-SE$_{bond}$
52.5 25.0 15.0 7.5 immediate
65.0 20.0 10.0 5.0 aged
CUB$_{LC}$+C-SE$_{bond}$
62.5 17.5 17.5 2.5 immediate
53.7 26.8 14.6 4.9 aged
SBU
72.5 20.0 7.5 immediate
72.5 15.0 10.0 2.5 aged
SBU$_{nonLC}$+C-SE$_{bond}$
51.2 24.4 14.6 9.8 immediate
72.5 17.5 5.0 5.0 aged
SBU$_{LC}$+C-SE$_{bond}$
57.1 21.4 14.3 7.1 immediate
60.0 20.0 15.0 5.0 aged
C-SE
74.0 6.0 20.0 immediate
62.0 10.0 24.0 4.0 aged
O-XTR
56.0 10.0 28.0 6.0 immediate
64.0 12.0 20.0 4.0 aged
0%
20%
40%
60%
80%
100%


a1
a2
dentin
CUB nonLC+C-SE bond immediate
adhesive resin
adhesive resin
Dentin side
Composite side
400 μm
500 μm
b1
b2
composite resin
composite resin
CUB LC+C-SE bond aged
adhesive resin
adhesive resin
dentin
Dentin side
Composite side
400 μm
400 μm
c1
c2
adhesive resin
adhesive resin
SBU nonLC+C-SE bond immediate
interface
Dentin side
Composite side
400 μm
400 μm
d1
d2
SBU LC+C-SE bond aged
interface
adhesive resin
Dentin side
Composite side
400 μm
400 μm