Extra bonding layer compensates universal adhesive's thin film thickness

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M.H. Ahmed: performed the experiments, statistical analysis, wrote the manuscript.

K. Van Landuyt: consulted on the idea, proofread the manuscript.

M. Peumans: consulted on the idea, proofread the manuscript.

B. Van Meerbeek: hypothesis, experimental design, and contributed substantially to the manuscript.

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ABSTRACT

Purpose: Universal adhesives (UAs) are applied in 2-step etch-and-rinse (2-E&R) or 1-step self-etch (1-SE) mode. This study investigated if three UAs could benefit from a highly filled extra bonding layer (EBL), turning them into 3-E&R and 2-SE UAs, respectively, hereby compensating for UAs' thin film thickness.

Materials and Methods: Micro-tensile bond strength (μTBS) to bur-cut dentin of Clearfil Universal Bond Quick (C-UBq; Kuraray Noritake), G-Premio Bond (G-PrB; GC) and Prime&Bond Active (P&Ba; Dentsply Sirona), applied in E&R and SE mode without/with the adhesive resin (EBL) of Optibond FL (Opti-FL_ar; Kerr), was compared to that of the 3-E&Ra Optibond FL (Opti-FL; Kerr), which was also employed in 2-SE mode. As cross-reference, the SE primer of Clearfil SE Bond 2 (Kuraray Noritake) was combined with Opti-FL_ar (C-SE2/Opti-FL) and again applied in 2-SE and 3-E&R mode. μTBS was measured upon 1-month (1m) water storage (37°C) and additional 25k and 50k thermocycles (TC). All μTBSs were statistically analysed using two different linear mixed-effects models with specific contrasts ($p<.05$).

Results: Overall, the four parameters ('Adhesive', 'Bonding mode', 'Aging', 'EBL') significantly influenced μTBS. G-PrB and P&Ba benefited from EBL when applied in both E&R and SE bonding modes. In E&R mode, P&Ba generally revealed the highest μTBS; C-UBq presented with an intermediate and G-PrB with the lowest μTBS. No significant differences were found between different bonding modes. C-SE2/Opti-FL outperformed Opti-FL in 3-E&R and 2-SE_1m/25k.

Conclusion: The overall benefit of EBL on the 1m and TC-aged bonding effectiveness differed for the specific UAs tested.

INTRODUCTION

Most of today's commercially available dental adhesives effectively bond to dentin at relatively short time, as evidenced by a favorable 'immediate' bonding effectiveness recorded in the laboratory.[8, 10] In laboratory research, all adhesives are most often bonded to near-ideal substrates under optimal *in vitro* conditions, this to determine the 'reference' bonding effectiveness of the adhesive tested. Current generation adhesives however still suffer from bond degradation upon accelerated artificial aging, being reflected in an 'aged' bond strength that is significantly lower than their respective immediate or reference bond strength.[10, 52] Not unimportantly, more reduction in bond strength was recorded for the adhesives that make use of simplified application procedures with combined primer/adhesive resin formulations and higher solvent content, this either when applied in a 2-E&R or 1-SE bonding mode.[8, 10]

The latest generation of universal adhesives (UAs) simplify the dentin-bonding process by enabling their optional applicability following either an 'etch-and-rinse' (E&R) or 'self-etch' (SE) bonding mode,[15] while the latter two approaches make use of two totally different adhesive mechanisms, each having their own capabilities and limitations.[8, 31, 43, 54] The term 'universal' has even more been expanded to self-adhesive composites.[56, 58]

Briefly, employing a UA in E&R bonding mode involves a phosphoric-acid etching as a first step followed by thorough water-rinsing prior to the application of a combined primer/adhesive resin in a 2-step procedure (2-E&R).[15, 51] Resin is expected to diffuse within the created micro-retention sites, by which this adhesive mode makes primarily use of a diffusion-based micro-mechanical interlocking mechanism. This mechanism is not always ideal, as the resultant several micrometers deep hybrid layer formed at dentin appeared sensitive to degradation with time. This bond degradation should primarily be attributed to incomplete saturation of the exposed collagen fibril network with resin, along with insufficient *in-situ* polymerization of the infiltrated resin.[51]

The SE bonding mode involves, on the other hand, the use of monomers with an acidic functional group rendering concurrent etching and tooth-substrate infiltration.[15, 28] The principal benefit of an SE bonding protocol is the additional chemical bonding potential. Using a 'mild' SE approach, dentin is only partially demineralized, by which a submicron hybrid layer is produced that still contains sufficient HAp for the functional monomer to ionically interact with.[7] The functional monomer 10-methacryloyloxydecyl dihydrogen phosphate (10-MDP) is today considered most effective,[43, 59] by which many commercially available UAs contain 10-MDP.[9] Major hurdle for an SE bonding protocol is potential interference of surface smear resulting from cavity preparation by bur.[13]

Many universal adhesives have been documented with effective 'immediate' laboratory and clinical performance, the latter with regard to the primary clinical parameters being restoration retention and marginal adaptation.[13] Nevertheless, a clear product dependency still exists for each bonding mode,

which necessitates further optimization in particular for 'long-term' clinical performance.[39, 52] A major reason that may limit bond durability of UAs, as well as of one-step adhesives, is their thin film thickness, often below 15 µm,[50] by which adequate polymerization of the adhesive layer is hampered by oxygen inhibition for a significant fraction of its depth (up to 12.5 µm).[30, 33] Along with the inherently higher hydrophilicity of UAs, water uptake is promoted, also rendering the bond more sensitive to monomer degradation/elution.[14] Additionally, a thin adhesive layer is even thought to reduce the adhesive layer's ability to absorb stress imposed to the adhesive interface, in particular polymerization-shrinkage stress induced by the overlaid composite restoration,[36, 53] which again may result in lower bonding effectiveness and promote bond degradation.[5]

In light of the previous rationale, several researchers advocated the use of an extra bonding layer (EBL) to improve the performance of UAs; this is based on both laboratory[38, 42] and clinical research.[26, 35] In this regard, a research project was initiated,[1] in which a representative selection of universal adhesives had been subjected to a micro-tensile bond-strength (µTBS) test to evaluate if they benefit from EBL with a higher hydrophobicity. This EBL was hypothesized to polymerize better and make the adhesive interface more resistant to bond-strength degradation. In a first project part, the adhesive resin of the gold-standard 2-SE adhesive Clearfil SE Bond 2 ('Bond'; Kuraray Noritake, Tokyo, Japan) was used as EBL.[1] In a side project in parallel with the first project part mentioned above, separate light-curing of the UA prior to the application and additional light-curing of EBL was compared to an application protocol, in which the UA was used as primer without separate light-curing prior to the application/light-curing of EBL.[14] In continuation of the first project part mentioned above,[1] the same UA selection was likewise tested with the highly particle-filled adhesive resin of the gold-standard 3-E&R adhesive Optibond FL (Kerr, Orange, CA, USA) employed as EBL. This EBL's nature with its high filler loading and high viscosity, automatically resulting in a thick adhesive-resin layer, is different from the lower silica-filled adhesive resin of Clearfil SE Bond 2 (Kuraray Noritake), and is well known for its superior mechanical properties such as lower polymerization shrinkage, different shrinkage kinetics, and higher elastic modulus.[24] The null hypotheses tested were that EBL has no effect on bonding effectiveness and bond durability of UAs when bonded to dentin either in E&R or SE bonding mode.

2. MATERIALS & METHODS

2.1. Dentin-specimen preparation

Forty non-carious human third-molars were collected (following informed consent approved by the Commission for Medical Ethics of the host institute under file number S54254) and stored in an aqueous solution of 0.5% chloramine T trihydrate powder at 4°C to prevent bacterial growth until use; all teeth were used within 1 month after extraction. Teeth were randomly divided into five

experimental groups of 8 teeth each. The occlusal third of the crowns were removed using a slow-speed diamond saw (Isomet 1000, Buehler, Lake Bluff, IL, USA) after mounting the teeth in gypsum blocks. Next, a uniform smear layer was produced on all mid-coronal dentin surfaces using a high-speed medium-grit (105 µm) diamond bur (F0142, Dentsply Sirona) mounted in a modified Micro-Specimen Former (University of Iowa, Iowa City, IA, USA). The exposed dentin surfaces were examined for absence of enamel and/or exposure of pulp tissue using a stereomicroscope (Wild M5A, Wild Heerbrugg, Heerbrugg, Switzerland).

The three UAs investigated in this study were Clearfil Universal Bond Quick ('C-UBq'; Kuraray Noritake), G-Premio Bond ('G-PrB'; GC, Tokyo, Japan) and Prime&Bond Active ('P&Ba'; Dentsply Sirona, Konstanz, Germany), which were applied in 3-E&R and 2-SE mode using the adhesive resin of Optibond FL (Opti-FL_ar; Kerr) as EBL. The gold-standard 3-E&Ra Optibond FL (Opti-FL; Kerr) served as reference, being also employed in an experimental 2-SE mode without beforehand phosphoric-acid etching. A cross-reference experimental group combined the SE primer of Clearfil SE Bond 2 (Kuraray Noritake) with Opti-FL_ar (C-SE2/Opti-FL), again applied both in 2-SE and 3-E&R mode. A split-tooth design was used with each adhesive applied in E&R mode on one tooth half and in SE mode on the other corresponding tooth half. Prior to phosphoric-acid etching (K-Etchant Syringe, Kuraray Noritake) as per instructions for use at the E&R tooth half, a single-edge carbon-steel blade (EMS, Hatfield, PA, USA) was positioned into a shallow 150-µm thick groove, splitting the dentin substrate in two halves to prevent acid leakage from the E&R to the SE tooth half. All UAs were applied as primer per instructions for use (without curing; Table 1) followed by EBL, which was light cured immediately upon its application. Each tooth was next restored in four composite (Clearfil AP-X, Kuraray Noritake; shade A3) increments to achieve a 5-6 mm build-up height. All light-curing in this study was carried out using the LED light-curing unit Bluephase 20i (Ivoclar Vivadent, Schaan, Liechtenstein) with a power output of at least 1500 mW/cm^2, as confirmed by a Marc Resin Calibrator (BlueLight Analytics, Halifax, Canada).

2.2. Micro-tensile bond-strength (µTBS) testing

Following light curing, the restored teeth were stored for one month (1m) in distilled water at 37°C. Next, the teeth were sectioned using a precision-cutting machine (Accutom 50, Struers, Ballerup, Denmark) to produce 1-mm^2 (±0.1 mm^2) sticks. Solely the six central micro(µ)-specimens were collected from each tooth half in order to minimize dentinal regional variability. The adhesive interface of all µ-specimens was examined for absence of enamel at the corners using a stereomicroscope (Wild M5A, Wild). The µ-specimens from each experimental group were randomly distributed into three thirds of 16 µ-specimens each. µTBS of one-third of the specimens was measured without any further thermocycling ('1m'), with the remaining two thirds aged by 25k and 50k thermocycles (TC; Thermocycler TH1200, SD Mechatronik, Munich, Germany).

The exact μ-specimen dimensions were measured using a 150-mm digital caliper (Digi-Met, Helios Preisser, Gammertingen, Germany), prior to fixing the specimens to BIOMAT jigs[41] with cyanoacrylate glue (Model Repair II Blue, Dentsply-Sankin, Ohtawara, Japan). The non-trimmed rectangular μ-specimens were stressed in tension using a universal testing device (LRX, Lloyd, Hampshire, UK) at a crosshead speed of 1 mm/min, upon which the μTBS was calculated in MPa by dividing the force imposed at the time of fracture by the bond area (mm^2). Any pre-test failures (ptfs) during cutting, storage, aging and/or jig mounting were explicitly recorded and included in the statistical analysis as 0 MPa. The μTBS protocol prescribed by the Academy of Dental Materials was strictly followed.[4] The UAs' reference μTBS, when applied without EBL following its respective manufacturer's instructions for use, were reported before.[1]

2.3. Failure analysis

All μ-specimen pairs (dentin and composite side) were examined after μTBS testing to determine the mode of failure ('mixed failure', 'cohesive failure in composite', 'adhesive interfacial failure', 'cohesive failure in dentin') using stereomicroscopy (Wild M5A, Wild). Furthermore, the failure mode of selected representative μ-specimen pairs (2-3 specimens/experimental group with a μTBS close to the mean; 2-3 ptfs, when available) were subsequently imaged using scanning electron microscopy (SEM; JSM-6610LV, Jeol, Tokyo, Japan) upon common SEM specimen processing, including fixation, gradual dehydration, chemical drying, and gold-sputter coating.[37] The failure analysis data of our previous work were also included.[1]

2.4. Statistical analysis

Statistical analysis was carried out using a linear mixed-effects (LME) statistical model with specific contrast (LME; R3.1.0; R Foundation for Statistical Computing, Vienna, Austria) to determine statistically significant differences at a significance level of α equaling 0.05. The previously published reference data recorded for the three UAs applied without EBL[1] were reinserted within the first statistical 'LME Model-1' to compare the EBL effect, with Opti-FL serving as reference for the statistical variables 'Adhesive', 'Bonding mode', 'Aging' and 'EBL'. Using the same statistical software, a second statistical 'LME Model-2' was constructed in order to statistically compare the cross-reference C-SE2/Opti-FL group, applied both in 2-SE and 3-E&R mode, with the gold-standard 3-E&Ra Opti-FL and the gold-standard 2-SEa C-SE2 (data reinserted),[1] both applied in their respective E&R and SE instructions-for-use bonding mode and their respective experimental SE and E&R counterpart mode. In addition, a third statistical model, namely 'LME Model-3', was constructed by including the data previously recorded for the three UAs applied without EBL, applied with C-SE2_ar[1] and

with Opti-FL_ar, but excluding the reference groups and the UAs_25k TC data in both studies. This LME Model-3 enabled testing which EBL the UAs investigated benefited most from. Using this LME model-3, also a more conclusive statistical analysis was carried out to assess the overall EBL benefit as well as the differential benefit from using C-SE2_ar or Opti-FL_ar as EBL.

RESULTS

The mean μTBS and fitted LME means are graphically presented in Figs. 1 and 2 for the three tested UAs with Opti-FL as reference, all applied with/without EBL in E&R mode in Fig. 1 and in SE mode in Fig. 2, and for Opti-FL, C-SE2 with C-SE2/Opti-FL as reference, all applied in both SE and E&R bonding mode in Fig. 3. All data are numerically detailed in Table 2. Statistically significant differences are mentioned in Figs. 1-3 with specific symbols that are explained in the respective graph.

Regarding LME Model-1, first-, second-, third- and fourth-order interactions were analyzed (Table 3). A fourth-order interaction (Adhesive:Aging:Bonding mode:EBL) was not found statistically significant and hence removed from further LME statistical analysis and fitted LME predictions. Despite being significant for all first-order interactions, none of the second-order interactions were found statistically significant, which means that any single variable did solely not modify any other variable. On the other hand, two third-order interactions, namely 'Adhesive:Bonding mode:EBL' and 'Adhesive:Aging:EBL' were found statistically significant, which means that, respectively, one or both bonding modes and one or more aging periods modified the effect of EBL on μTBS of one or more adhesives included in this model.

In LME Model-1 regarding the parameter 'adhesive' (without EBL) as compared to the reference Opti-FL (statistical difference indicated by 'X' in Figs. 1 and 2), P&Ba revealed significantly higher μTBS than Opti-FL when applied in E&R mode, except after 50k TC, and when applied in SE mode only after 1m water storage. After 50k TC, GPrB showed significantly lower μTBS than Opti-FL when applied in SE mode. For all other conditions, the UAs did not under/overperform Opti-FL. When EBL was employed (LME Model-1; Figs. 1 and 2), C-UBq with EBL revealed only one time (1m in SE mode) a significantly higher μTBS than Opti-FL. G-PrB with EBL outperformed Opti-FL only two times (1m in E&R and SE mode). Nevertheless, all bonding modes and aging regimes resulted in significantly higher μTBSs than Opti-FL when P&Ba was used as primer and followed by EBL.

In LME Model-1 regarding the parameter 'EBL' as compared to the respective adhesive's μTBS obtained by application without EBL (statistical difference indicated by '★' in Figs. 1 and 2), no benefit of EBL was recorded when the UAs were applied in E&R bonding mode, except for G-PrB and P&Ba that benefited from EBL in 1m/50k and 25k/50k, respectively. In SE bonding mode, C-UBq_1m/50k and

G-PrB_1m/25k/50k (all 3 conditions) benefited from EBL. In LME Model-1 regarding the parameter 'aging', as having compared the μTBS measured after 25k and 50k TC to the 1m μTBS (statistical difference indicated by '#' in Figs. 1 and 2), solely the G-PrB_50k_EBL μTBS was statistically significantly lower than the 1m μTBS when applied in SE bonding mode. In LME Model-1 comparing the E&R and SE bonding modes of the respective experimental groups, no statistically significant differences were recorded.

In LME Model-2 comparing the 2-SEa C-SE2 and 3-E&Ra Opti-FL with the cross-reference C-SE2/Opti-FL applied in both SE and E&R mode, none of the adhesives received EBL, by which solely three parameters ('adhesive', 'bonding mode', 'aging') were tested. Significant first-, second- and third-order interactions were proposed. All interactions were hence kept within the statistical model and included for subsequent LME predictions. In addition, all three parameters were found significant when tested in first-order interaction. However, the second-order interaction 'Bonding mode:Aging' and the third-order interaction 'Adhesive:Bonding mode:Aging' were not found statistically significant.

In LME Model-2 regarding the parameter 'adhesive' as compared to the cross-reference C-SE2/Opti-FL μTBS (statistical difference indicated by 'X' in Fig. 3), Opti-FL_3-E&R_1m/25k and Opti-FL_2-SE_1m revealed a significantly lower μTBS than that of C-SE2/Opti-FL. No significant difference in μTBS was recorded between C-SE2_3-E&R/2-SE and C-SE2/Opti-FL. Additionally, the LME model revealed significantly higher μTBS for C-SE2 than Opti-FL in all experimental groups except after 50k TC when both adhesives were employed in SE mode (indicated by '★' in Fig. 3). In LME Model-2 regarding the parameter 'aging' as having compared the μTBS measured after 25k and 50k TC to the 1m μTBS (statistical difference indicated by '#' in Fig. 3), solely C-SE2's μTBS significantly decreased after 25k and 50k TC when employed in E&R mode and after 50k TC when employed in SE bonding mode. Regarding the parameter 'bonding mode', comparing the SE and E&R μTBS (statistical difference indicated by '∗' in Fig. 3), solely the C-SE2_1m μTBS was significantly higher when employed in E&R than SE bonding mode.

Regarding LME Model-3, overall a significantly higher μTBS was recorded for the UAs applied with EBL (versus without EBL), this when measured at 1m and after 50k TC (Fig. 4a). While no statistical difference in μTBS was recorded between the UAs applied with C-SE2_ar and Opti-FL_ar at 1m, the UAs benefited significantly more from Opti-FL_ar (than C-SE2_ar) after 50k TC. Considering each bonding mode separately (Fig. 4b), when the UAs were applied in E&R mode, they statistically significantly benefited solely from Opti-FL_ar applied as EBL upon aging after 50k TC, while when they were applied in SE Mode, they statistically significantly benefited from both EBLs, except not significantly from C-SE2_ar upon aging after 50k TC. Considering the UAs individually (Fig. 5), C-UBq benefited least from EBL, with solely a significantly higher μTBS recorded for C-UBq_1m_Opti-FL_ar in SE mode. G-PrB benefited most from all UAs investigated, with significantly higher μTBS recorded when

Opti-FL_ar was additionally applied as EBL in all experimental conditions and with also significantly higher μTBS recorded when C-SE2_ar was additionally applied as EBL in SE mode both at 1m and after 50k TC. P&Ba benefited solely from Opti-FL_ar after 50k TC when applied both in E&R and SE mode.

Overall, pre-test failures were solely recorded in a relatively low number in different experimental groups without a consistent tendency noted for any of the experimental groups (Table 2). No pre-test failures were recorded for the gold-standard adhesives Opti-FL and C-SE2, while solely 1 pre-test failure was recorded in the 1m and 50k TC experimental group for the cross-reference C-SE2/Opti-FL. Fractographic analysis revealed that most failures were recorded as adhesive interfacial failures (Figs. 6 and 7). In general, higher μTBSs were associated with a higher tendency to fail cohesively within dentin/composite or mixed, as particularly recorded for P&Ba applied in all 2/3-E&R and1/2-SE bonding modes, for C-UBq applied in 3-E&R mode, for C-SE2 applied in 2-E&R and 1-SE mode, and for C-SE2/Opti-FL in 3-E&R and 2-SE mode. Almost all G-PrB experimental groups revealed adhesive interfacial failures for both the E&R and SE bonding modes. SEM examination of representative fractured surfaces revealed that EBL remained bonded to the adhesive layer in most of the 0 TC (1m) specimens, but partially to completely de-bonded from the adhesive layer for the 'aged' 25k and 50k TC specimens (Figs. 8-12).

DISCUSSION

In order to improve bonding effectiveness of simplified adhesives, including UAs, several approaches to retard/prevent bond degradation were suggested in literature.[6, 25, 32, 48] In light of the current work, some studies recommended applying single-step adhesives in sufficient layers, hereby always more than the minimum number of layers recommended by the manufacturer, so to produce a thicker adhesive film.[3, 47] Others suggested the application of a separate and additional hydrophobic layer to improve bond durability by sealing the adhesive interface better against water uptake.[3, 29, 38, 42, 44] Achieving a thicker film thickness may indeed be advantageous, as the application of a particle-filled low-viscosity resin in between the hybrid layer and the shrinking composite has been shown to act as an elastic cavity wall or shock absorber of stresses imposed to the adhesive interface, this to the benefit of the adhesive restoration's marginal adaptation and retention.[19, 36, 53] The newest UA generation, being the focus of this study, make use of combined primer/adhesive resin one-bottle solutions that are generally more hydrophilic and proportionally contain more solvent, by which their film thickness remains relatively thin (Table 5).[33]

In light of the abovementioned challenges and strategies, we tested in our previous study if three UAs benefited from C-SE2's adhesive resin ('C-SE2_ar'; Clearfil SE Bond 2 Bond, Kuraray Noritake) applied as an extra bonding layer (EBL) on top of the three representative UAs C-UBq, G-PrB and

P&Ba.[1] Mixed results were obtained concluding that the overall effect of EBL on immediate and aged bonding effectiveness depended on the specific UA and its bonding mode. In continuation of the previous study,[1] the potential benefit of Opti-FL_ar as EBL on immediate and aged bonding effectiveness of the same three UAs was investigated in this study. Opti-FL_ar is a hydrophobic, solvent-free adhesive resin that is highly filled with a mixture of barium-aluminum borosilicate glass, disodium hexafluorosilicate and fumed silica up to a filler load of 48 wt%. The adhesive is rather viscous (substantially more than other commercial adhesives and certainly UAs), by which a relatively high film thickness of about 50 µm[46, 50] is typically obtained upon its application (Table 5). Because of this high film thickness, Opti-FL_ar was made radiopaque. As Opti-FL has repeatedly and consistently been documented with favorable laboratory[11] and clinical data,[40] its adhesive resin (Opti-FL_ar) must at least in part have contributed to Opti-FL's well documented predictable and reliable bonding performance. Like in the previous study, the UAs investigated were used as primers without being separately light-cured, hereby having transformed them into 3-E&R and 2-SE adhesives depending on the respective adhesive mode employed; the adhesive interface was adequately light-cured upon application of Opti-FL_ar (EBL). The gold-standard 3-E&R adhesive Optibond FL (Opti-FL) served as reference adhesive and was also applied in an experimental 2-SE mode, considering that Opti-Fl Prime contains the functional monomer glycero-phosphate dimethacrylate (GPDM) and in fact can be regarded as a self-etch primer[16, 61] with a pH of about 2.[21]

For profound statistical analysis in this study, the extensive comparison between the different experimental groups required linear mixed-effects (LME) modelling that additionally considered inter-tooth differences which originate from the split-tooth design with the UAs applied on each tooth in both an E&R and SE bonding mode.[27] Differently from our previous work,[1] this study design involved two statistical LME models. The first statistical LME Model-1 involved 4 parameters: (1) 'Adhesive' with three levels corresponding to the three UAs investigated; (2) 'Bonding mode' with two levels, namely E&R and SE; (3) 'Aging' with three levels, namely 1m (0 TC), and additional 25k and 50k TC; and (4) 'EBL' with two levels, namely without and with EBL. In order to test the effect of EBL separately, the µTBS data previously reported[1] for the three UAs applied without EBL were included in the current LME Model-1, so to be able to compare the non-EBL µTBS data to the EBL µTBS data of the corresponding experimental groups in the current study. The second LME Model-2 was next constructed in order to compare the gold-standard E&R adhesive Opti-FL, which is the reference adhesive in this study, and the gold-standard SE adhesive C-SE2, which was the reference adhesive in the previous study,[1] with the cross-reference adhesive CSE2/Opti-FL. The latter experimental adhesive combination was selected, hypothesizing to combine one of the best performing commercial primers containing 10-MDP, as also contained in many UAs, with one of the best performing adhesive resins. LME Model-2 involved 3 parameters: (1) 'Adhesive' with three levels, namely Opti-FL, C-SE2 and

CSE2/Opti-FL; (2) 'Bonding mode' with two levels, namely E&R and SE; and (3) 'Aging' with three levels, namely 1m (0 TC), and additional 25k and 50k TC.

Regarding the UA P&Ba, the experimental 3-E&R application mode, including EBL, generally outperformed the manufacturer's instructed 2-E&R application mode, even statistically significant after 25k and 50k TCs (Fig. 1). P&Ba's composition is relatively highly hydrophilic, enabling good wetting potential at dentin with different surface-wetness degrees, a major advantage claimed by its manufacturer.[12] The drawback of a high hydrophilic nature is the expected higher water sorption sensitivity, as was recorded in a separate project for P&Ba.[55] The significantly better aged bonding performance of P&Ba with EBL may indicate that the additional application of the hydrophobic Opti-FL_ar EBL may have reduced water sorption and thus hydrolytic degradation upon medium/long-term TC, a finding that was however not recorded when C-SE2_ar was applied as EBL in our previous work.[1] However, when P&Ba was applied in SE mode, no statistically significant benefit from EBL was obtained (Fig. 2). In principle, E&R adhesive interfaces, involving a thicker hybrid layer as well as tubules widely opened by phosphoric acid, are more permeable than SE interfaces, at which the hybrid layer is thinner and still rich in hydroxyapatite, along with dentin tubules that remained more closed due to the significantly milder SE treatment.[54] This reasoning may explain why EBL was found less beneficial at SE than E&R interfaces produced by P&Ba. The same reason explains the typically higher post-operative sensitivity recorded for E&R adhesives as compared to that of SE adhesives.[34, 52] Otherwise, being an E&R adhesive, the low post-operative sensitivity typically recorded for Opti-FL may also be ascribed to the separate application of the thick Opti-FL_ar in its 3-step application procedure.[31, 50]

On the other hand, and more difficult to explain, P&Ba applied with/without EBL generally resulted in significantly higher μTBS than the reference Opti-FL (solely not for three experimental groups in Figs. 1 and 2), which was not expected. Different from our previous work when C-SE2_ar was used as EBL, no bond-strength reduction upon TC was recorded when P&Ba was followed by Opti-FL_ar as EBL in this study. A plausible explanation might be the lower water sorption and thus lower hydrolytic degradation sensitivity of Opti-FL_ar than of C-SE2_ar, which needs to be confirmed in continued research.

Regarding the UA G-PrB, among all UAs tested in the study, the EBL benefit was most clear when Opti-FL_ar was applied following G-PrB used as primer, this both in E&R and SE mode; μTBS of solely one experimental group (G-PrB_E&R_25k) was not statistically higher than its EBL-free counterpart (Figs. 1 and 2). This finding confirms the findings when C-SE2_ar was applied as EBL in our previous study.[1] G-PrB contains besides 10-MDP also a second functional monomer 4-methacryloxyethyl trimellitic acid (4-MET), by which G-PrB is significantly more acidic (pH≈1.5)[16] than the other two UAs (pH>2.5) (Table 1). G-PrB should therefore be categorized as an 'intermediary strong' UA/SE adhesive, while C-UBq and P&Ba should be regarded as 'ultra-mild' UA/SE adhesives.[42] Succeeding its

commercial pre-cursors G-Bond (GC) and G-aenial Bond (GC; G-Bond Plus in specific world parts), 4-MET was added to G-PrB with the intention to (self-)etch enamel better and thus to achieve better bonding effectiveness at enamel when applied in (full) SE mode. Although 4-MET was generally thought to etch hydroxyapatite substrates and release Ca less than 10-MDP, more recent research demonstrated the contrary that 10-MDP etches and releases more Ca than 4-MET.[60] 10-MDP also outperforms 4-MET in terms of higher chemical bonding potential and production of more stable monomer-Ca salts, the latter additionally resulting in 10-MDP's characteristic nano-layering, both thought to contribute to bond stability.[59, 63] Following a meta-analysis conducted by Cuevas-Suárez *et al.*,[9] 'intermediary strong' adhesives, such as G-PrB, presented with lower bond strength and higher bond-strength reduction upon aging, irrespective of the bonding mode used.

Regarding the UA C-UBq, its performance among the three UAs studied in this study generally appeared to have resulted in intermediate bond-strength results, similarly to what was found in our previous study using C-SE2_ar as EBL.[1] In total, only statistical significances for three experimental conditions were found (Figs. 1 and 2). Basically, no significant difference in µTBS was recorded between C-UBq and the Opti-FL reference applied both in E&R and SE mode (except for C-UBq_SE_1m; Figs. 1 and 2). Most striking was that EBL resulted in significantly higher µTBS when C-UBq was used in SE mode (solely not for C-UBq_SE_25k in Fig. 2). C-UBq features a new hydrophilic acrylamide monomer, by which the HEMA content could be reduced (2.5-10% according to C-UBq's Material Safety Data Sheet),[22] as compared to earlier Clearfil (Kuraray Noritake) adhesive generations.[23] Associated with this reduced HEMA content, water sorption is claimed to be reduced and polymerization conversion improved. Nevertheless, application and direct light-curing without waiting of C-UBq (so-called 'rapid bond technology') is perhaps more a marketing advantage (in particular in Japan) than a true benefit, as this relatively small time saving may clinically be less relevant.[2] Like P&Ba, C-UBq, which contains solely 10-MDP as functional monomer, is less acidic and thus should be regarded as an 'ultra-mild' UA formulation with potentially higher stability upon aging.[9]

Comparing the gold-standard SE and E&R adhesives, C-SE2 outperformed, somewhat unexpectedly, Opti-FL when both were applied in a 3-E&R bonding protocol (statistically different for all experimental conditions in Fig. 3). Before, a dedicated SE adhesive was discouraged to be applied on beforehand phosphoric-acid etched dentin, hereby losing all chemical bonding potential to hydroxyapatite, which is completely dissolved by phosphoric acid and removed upon water rinsing, this up to a depth of 4-5 µm.[49] The latter study by Van Landuyt *et al.* in 2006 investigated two specifically formulated experimental one-step adhesives, modifying them into 2-SEa's and 3-E&Ra's,[49] by which the results cannot directly be extrapolated to other adhesive formulations. This study on the contrary showed that C-SE2 can also effectively be used in E&R mode, like a UA, as it in this study appeared to have even better diffused into the exposed collagen fibril network during the E&R hybridization process than

Opti-FL, with diffusion-based micro-mechanical interlocking being the principal bonding mechanisms employed by E&R adhesives.[42]

When applied in a 2-SE bonding protocol, C-SE2 outperformed, as expected, Opti-FL (statistically significantly different for two out of the three experimental conditions in Fig. 3). This should primarily be ascribed to the high chemical bonding potential of 10-MDP,[59] contained in a high quality/purity in C-SE2.[62] Opti-FL contains GPDM as functional monomer (Table 1). Although GPDM was documented to be adsorbed onto hydroxyapatite, it was found incapable of forming a stable monomer-Ca salt.[61] Moreover, the bond between GPDM and hydroxyapatite appeared to be weak, unlike the strong bond formed by 10-MDP to hydroxyapatite.[61] Due to its high hydrophilicity, GPDM is nevertheless thought to be an adequate functional monomer to be contained in an E&R adhesive, but it appears less appropriate for a '(ultra-)mild' SE adhesive that besides micro-retention ionically interacts with hydroxyapatite (or even for a self-adhesive restorative material). These fundamental mechanistic data support/explain the found better SE performance of C-SE2 as compared to that of Opti-FL. Only upon 50k TC, the μTBS of C-SE2 applied in 2-SE mode decreased, so that a statistically different aged bonding effectiveness was no longer recorded for C-SE2 as compared to the equivalent μTBS measured for Opti-FL, also applied in 2-SE mode. Despite 10-MDP's high primary chemical bonding potential,[59] such reduced bonding effectiveness of C-SE2 upon aging has been recorded before.[45, 57] Enhanced water sorption with a plasticizing effect, hereby reducing the adhesive's intrinsic mechanical strength with time, may be a plausible explanation for the observed bond-strength decrease of C-SE2 upon aging (observed when applied both in SE and E&R mode). Such bond-strength reduction did not occur with Opti-FL, perhaps thanks to the more hydrophobic nature and high filler loading of Opti-FL_ar.

Statistical comparison of the benefit the UAs obtain from applying one of the two EBLs investigated in this study (Opti-FL_ar) and the previous study (C-SE2_ar) confirmed the previously addressed findings. Overall, EBL significantly improved bonding effectiveness of the UAs combined, this relatively immediately, as measured in both complementary studies at 1m, and upon aging after 50k TC. Considering each bonding mode separately, when the UAs were applied in E&R mode, they statistically significantly benefited solely from Opti-FL_ar applied as EBL upon aging after 50k TC, while when they were applied in SE Mode, they statistically significantly benefited from both EBLs, except not significantly from C-SE2_ar upon aging after 50k TC. Comparing both EBLs investigated in this study (Opti-FL_ar) versus that in the previous study (C-SE2_ar), no statistical difference in overall μTBS was found at 1m, while this difference was significant after 50k TC. Most likely, this statistical difference in contribution of both EBLs to aged bonding effectiveness should be attributed to their difference in EBL nature. In order to understand the impact of this different EBL nature on bond durability, more basic knowledge on mechanical properties of the fully polymerized EBLs would be very informative. Previous work by our group revealed that the elastic modulus of the 48 wt% particle-filled Opti-FL_ar (9.3 GPa)

was significantly higher than that of the low silica-filled adhesive resin (4.8 GPa) of Clearfil Liner Bond 2 (Kuraray Noritake), being a precursor of C-SE2_ar.[24] That paper already then mentioned that studies indicated that thicker adhesive-resin layers were associated with lower interfacial stresses and better preserved marginal adaptation.[19] A concern raised was that if an adhesive had to be chosen for its ability to be placed in thicker layers, such as the particle-filled Opti-FL_ar, this adhesive may not be a better stress buffer because of its significantly higher rigidity.[24] It was however also stated that the stress-relieving effects of a manifold increase of the adhesive-resin layer thickness may largely overcome the opposite influence of a higher elastic modulus. Related research reported on the fracture strength of adhesives and their importance with regard to bond strength revealed no difference in 24-hour micro-tensile fracture strength (μTFS) between Opti-FL_ar and the adhesive resin of Clearfil SE Bond (Kuraray Noritake), as the immediate precursor of Clearfil SE Bond 2 (Kuraray Noritake) studied in this paper, this despite the significantly higher filler content of Opti-FL_ar.[18,20] Even more, the 1-hour μTFS of the adhesive resin of Clearfil SE Bond (Kuraray Noritake) exceeded significantly that of Opti-FL_ar, indicating that the adhesive resin of Clearfil SE Bond (Kuraray Noritake) must have polymerized substantially more efficiently than Opti-FL_ar. On the other hand, the μTFS of the respective primer-adhesive mixtures (1:3 ratio) was significantly reduced as compared to that of the pure adhesive resins. While no difference in 1-hour μTFS was recorded, the 24-hour μTFS of the Opti-FL primer-adhesive mixture was significantly higher than that of the Clearfil SE Bond (Kuraray Noritake) primer-adhesive mixture, indicating that the adhesive Clearfil SE Bond (Kuraray Noritake) was mechanically more affected by the primer than Opti-FL_ar. This difference was ascribed to different kind and concentration of solvents within the respective primers (Opti-FL primer: ethanol, water; Clearfil SE Bond primer (Kuraray Noritake): water), different evaporation of primer solvents and differences in the resultant residual water content in the respective primer-adhesive mixtures.[17,18] Hence multiple parameters play a role, by which further parameter-controlled research is needed to define the optimum EBL composition.

We additionally compared the bonding effectiveness of an interesting combination of C-SE2 Primer with Opti-FL_ar, when applied both in E&R and SE bonding mode. As mentioned above, the rationale behind testing the experimental combination of a commercial primer with a commercial adhesive resin from two different companies, is that both adhesives are considered as gold-standard in their SE and E&R adhesive class, respectively. Consequently, both adhesives most likely provide, respectively, the most optimum 10-MDP-based primer ('C-SE2 Primer', Kuraray Noritake) and the most optimum highly particle-filled and solvent-free hydrophobic adhesive resin ('Opti-FL Adhesive', Kerr), which are today commercially available. This study proved that this experimental C-SE2/Opti-FL cross-reference performed in E&R mode better (significantly in two of the three experimental conditions) than Opti-FL and equally effective as C-SE2. In SE mode, C-SE2/Opti-FL generally performed as good as C-SE2 and

Opti-FL, the latter except for Opti-FL_2-SE_1m that resulted in a significantly lower μTBS than that of the equivalent experimental C-SE2/Opti-FL group.

All the findings discussed above can be correlated to the LM and SEM fractographic analysis. It is very clear that the predominant failure mode in the current study was interfacial, irrespective of the variables 'adhesive', 'bonding mode', 'EBL', or 'aging'. SEM examination revealed a detailed explanation of the failure modes recorded in the experimental groups when EBL was employed. In some experimental groups, such as P&Ba_2-SE, EBL was observed to have remained attached to the dentin side even after 50k TC. This observation highlights the protective effect a thick EBL can provide to adhesive-dentin interfaces.

Finally, the null hypotheses that EBL has no effect on bonding effectiveness and bond durability of UAs when bonded to dentin either in E&R or SE bonding mode, failed to be rejected for some experimental groups. Nevertheless, in summary and conclusion, UAs overall seemed to benefit from EBL, considering the higher bond strength most often recorded when the UAs were applied as primer and followed by the EBL. Overall, the highly particle-filled hydrophobic adhesive Opti-FL_ar, hereby having generated a thicker film thickness, overperformed the low silica-filled C-SE2_ar.

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Tables

Table 1. Chemical composition and instructions for use of the materials used.

Material Code Lot number	Composition	pH	Instructions for use
Clearfil Universal Bond Quick (Kuraray Noritake, Tokyo, Japan) **C-UBq** AQ0009	Bis-GMA (10-25%), HEMA (2.5-10%), 10-MDP, hydrophilic amide monomer, colloidal silica, silane coupling agent, sodium fluoride, camphorquinone, ethanol (10-25%), water	2.7	**SE:** Apply in rubbing motion, no waiting, mildly air-dry (≥5 s) until the adhesive does no longer move, light-cure (10 s). **E&R:** Etch for 10 s with K-Etchant Syringe (Kuraray Noritake), thoroughly water rinse (>10 s), gently air-dry, then proceed as for SE.
G-Premio Bond (GC, Tokyo, Japan) **G-PrB** 1606281	10-MDP (5-10%), 4-MET, dimethacrylate (10-20%), dimethacrylate component (1-5%), photo initiator (1-5%), butylated hydroxytoluene (<1%), acetone (25-50%), water (24%)	1.5	**SE:** Apply and wait for 10 s, dry thoroughly with maximum air pressure (10 s), light-cure (10 s). **E&R:** Etch for 10 s with K-Etchant Syringe (Kuraray Noritake), thoroughly water rinse (>10 s), gently air-drying, then proceed as for SE.
Prime&Bond Active (Dentsply Sirona, Konstanz, Germany) **P&Ba** 1609000096	Bisacrylamide 1 (25-50%), 10-MDP (10-25%), bisacrylamide 2 (2.5-10%), 4-(dimethylamino) benzonitrile (0.1-1%), PENTA, propan-2-ol (10-25%), water (20%)	2.5	**SE:** Apply adhesive, slightly agitate (20 s), mildly air-dry (>5 s), light-cure (10 s). **E&R:** Etch for 10 s with K-Etchant Syringe (Kuraray Noritake), thoroughly water rinse (> 10 s), gently air-dry, then proceed as for SE.
Optibond FL (Kerr, Orange, CA, USA) **Opti-FL** 000041 EBL: 8R0074	*Prime:* HEMA, ethanol, 2-[2-(methacryl-oyloxy) ethoxycarbonyl] benzoic acid, GPDM *Adhesive: barium-aluminum borosilicate* glass, fumed silica, HEMA, ytterbium trifluoride, trimethoxy-silylpropyl methacrylate, 2-hydroxy-1,3-propanediyl, bismethacrylate, disodium hexfluorosilicate	2	**SE:** Apply *Prime* with a light scrubbing motion (15 s), gently air-dry (5 s); Apply *Adhesive*, gently air-dry to make a uniform layer, light-cure (10 s). **E&R:** Etch for 10 s with K-Etchant Syringe (Kuraray Noritake), thoroughly water rinse (>10 s), gently air-dry, then proceed as for SE.
K-Etchant Syringe (Kuraray Noritake) 5x0696	Phosphoric acid (35-45%)	<1	Etch for 10 s, thoroughly water rinse (>10 s), gently air-dry.
Clearfil AP-X (Kuraray Noritake) 6E0696	Bis-GMA (<12%), TEG-DMA (<5%), silanated barium glass filler, silanated silica filler, silanated colloidal silica, dl-camphorquinone, catalysts, accelerators, pigments (shade: A3)	NA	One compule per increment (1.5-mm height), light-cure (10 s), apply 4 increments (6-mm height), then light-cure (10 s) from each side.

Abbreviations in alphabetical order: ***Bis-GMA*** *= bisphenol A diglycidyl methacrylate,* ***GPDM****= glycero-phosphate dimethacrylate,* ***HEMA*** *= 2-hydroxyethyl methacrylate,* ***PENTA*** *= dipentaerythritol pentacrylate phosphate;* ***TEG-DMA*** *= triethylene glycol dimethacrylate;* ***4-MET*** *= 4-methacryloxyethyl trimellitic acid;* ***10-MDP*** *= 10-methacryloyloxydecyl dihydrogenphosphate.*

Table 2. Mean μTBS and fitted LME means for all the different E&R and SE experimental groups (in MPa).

BONDING MODE	E&R		SE	
Experimental group	**Mean ± SD (ptf/n)**	**Fitted LME mean**	**Mean ± SD (ptf/n)**	**Fitted LME mean**
C-UBq_1m	33.03±09.93 (0/16)	33.24	22.19±08.24 (0/16)	21.97
C-UBq_25k TC	30.89±16.28 (1/16)	31.39	23.80±11.96 (0/16)	23.31
C-UBq_50k TC	25.77±17.40 (3/16)	25.07	16.19±11.98 (5/16)	16.90
C-UBq_1m_EBL	33.46±17.41 (1/16)	33.25	38.04±17.81 (0/16)	38.25
C-UBq_25k TC_EBL	31.83±15.81 (2/16)	31.34	33.41±16.21 (1/16)	33.90
C-UBq_50k TC_ EBL	34.31±22.68 (2/16)	35.02	32.70±10.75 (0/16)	31.99
G-PrB_1m	21.82±10.84 (0/16)	23.35	16.91±07.33 (0/16)	15.38
G-PrB_25k TC	18.81±09.98 (2/16)	17.93	16.36±06.57 (0/16)	17.24
G-PrB_50k TC	16.69±09.55 (2/16)	16.04	09.56±06.84 (4/16)	10.37
G-PrB_1m_EBL	42.16±20.93 (0/16)	40.63	40.66±15.86 (1/16)	42.20
G-PrB_25k TC_EBL	30.04±25.14 (2/16)	30.92	35.03±15.70 (2/16)	34.15
G-PrB_50k TC_EBL	33.77±22.77 (3/16)	34.42	27.84±18.69 (4/16)	27.19
P&Ba_1m	47.35±16.28 (0/16)	43.74	38.47±19.65 (2/16)	42.07
P&Ba_25k TC	44.09±19.37 (1/16)	45.17	40.20±18.02 (1/16)	39.12
P&Ba_50k TC	28.87±21.01 (5/16)	31.39	44.04±17.74 (0/16)	41.51
P&Ba_1m_EBL	47.92±15.08 (0/16)	51.52	52.14±16.80 (0/16)	48.53
P&Ba_25k TC_EBL	61.05±20.02 (0/16)	59.96	45.88±19.13 (0/16)	46.96
P&Ba_50k TC_EBL	59.65±21.44 (0/16)	57.13	52.29±19.11 (0/16)	54.81
Opti-FL_1m	26.71±16.89 (0/16)	27.79	22.84±17.97 (3/16)	21.76
Opti-FL_25k TC	24.63±15.05 (0/16)	24.32	26.81±13.15 (0/16)	27.12
Opti-FL_50k TC	28.94±12.66 (0/16)	28.17	25.88±14.64 (2/16)	26.65
C-SE2_1m	60.67±17.14 (0/16)	59.43	43.83±17.43 (0/16)	45.07
C-SE2_25k TC	48.45±13.53 (0/16)	47.72	41.47±15.70 (0/16)	42.20
C-SE2_50k TC	38.92±12.94 (0/16)	40.89	33.01±10.48 (0/16)	31.04
C-SE2/Opti-FL_1m	53.20±24.59 (1/16)	53.36	39.15±15.08 (1/16)	38.99
C-SE2/Opti-FL_25k TC	41.64±12.27 (0/16)	42.68	38.19±18.77 (1/16)	37.14
C-SE2/Opti-FL_50k TC	38.47±16.83 (1/16)	37.27	26.20±14.03 (2/16)	27.40

***SD** = Standard deviation; **ptf** = Pre-test failure; **n** = Total specimen number.*

Table 3. LME Model-1 statistical analysis for the first-, second-, third- and fourth-order interactions ($p<0.05$).

	numDF	denDF	F-value	p-value
(Intercept)	1	670	1697.2	<.0001*
Adhesive	3	56	31.266	<.0001*
Bonding mode	1	670	10.721	0.0011*
Aging	2	670	5.5428	0.0041*
EBL	1	56	65.397	<.0001*
Adhesive:Bonding mode	3	670	0.3895	0.7606
Adhesive:Aging	6	670	0.8198	0.5547
Adhesive:EBL	3	56	1.4316	0.2432
Bonding mode:Aging	2	670	0.1483	0.8622
Bonding mode:EBL	1	670	0.0043	0.9476
Aging:EBL	2	670	0.6962	0.4988
Adhesive:Bonding mode:EBL	3	670	3.9154	0.0087*
Adhesive:Bonding mode:Aging	6	670	1.6963	0.1192
Adhesive:Aging:EBL	6	670	2.6013	0.0169*
Bonding mode:Aging:EBL	2	670	1.9573	0.1420
Adhesive:Bonding mode:Aging:EBL	6	664	1.2777	0.2653

***** *Statistically significant.*

Table 4. LME Model-2 statistical analysis for the first-, second- and third-order interactions ($p<0.05$).

	numDF	denDF	F-value	p-value
(Intercept)	1	253	1010.5890	<.0001*
Adhesive	2	21	22.8750	<.0001*
Bonding mode	1	253	15.5138	0.0001*
Aging	2	253	8.5445	0.0003*
Adhesive: Bonding mode	2	253	2.3514	0.0973*
Adhesive:Aging	4	253	3.6020	0.0071*
Bonding mode:Aging	2	253	1.9809	0.1401
Adhesive: Bonding mode:Aging	4	249	0.3467	0.8462

***** *Statistically significant.*

Table 5. Film thickness (in µm) of the adhesive resin layer for the different adhesives investigated.

Adhesive	Adhesive layer film thickness
C-UBq[2]	8-10
G-PrB[55]	2-3
P&Ba*	8-10
Opti-FL[50]	50-100
C-SE2[2]	12-15

** Unpublished TEM observations.*

FIGURES

FIG. 1

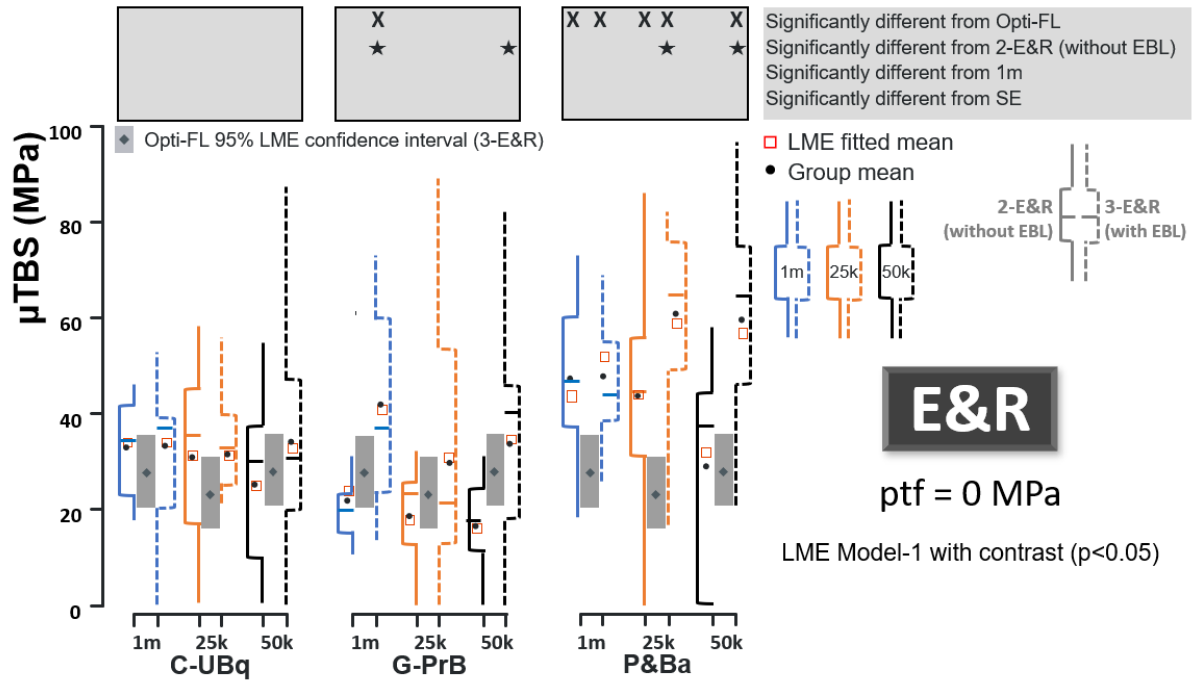

FIG. 2

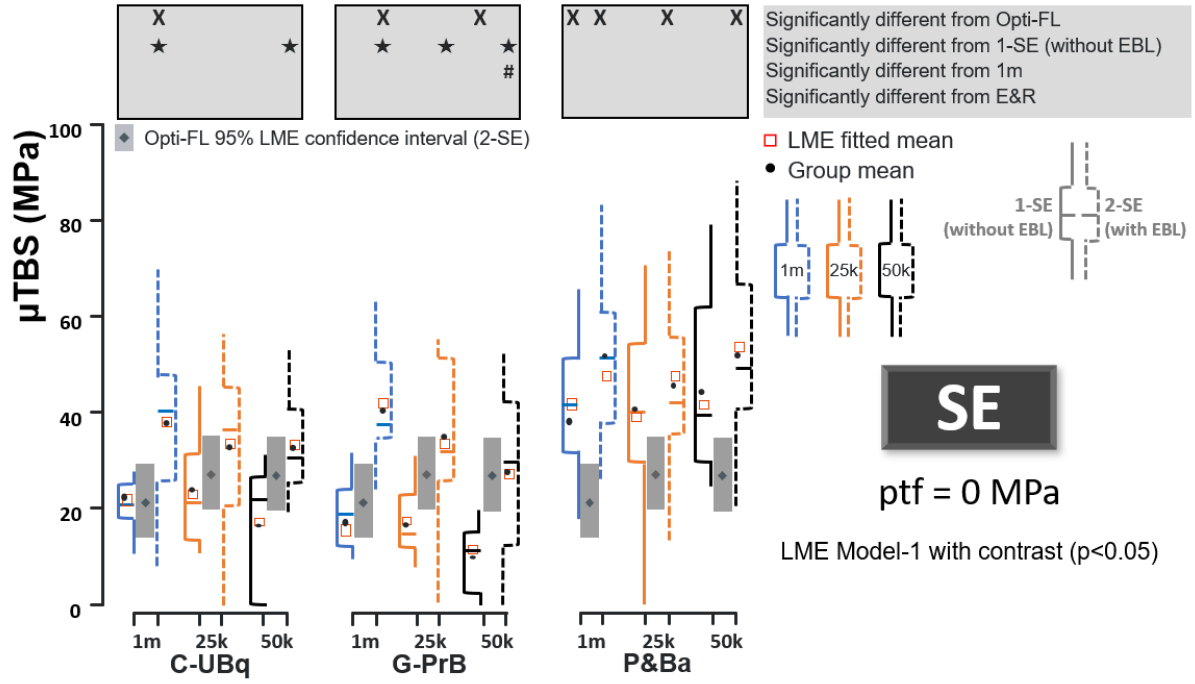
Significantly different from Opti-FL
Significantly different from 1-SE (without EBL)
Significantly different from 1m
Significantly different from E&R
Opti-FL 95% LME confidence interval (2-SE)
LME fitted mean
Group mean
1-SE (without EBL)
2-SE (with EBL)
1m
25k
50k
SE
ptf = 0 MPa
LME Model-1 with contrast (p<0.05)
µTBS (MPa)
100
80
60
40
20
0
1m
25k
50k
C-UBq
G-PrB
P&Ba

FIG. 3

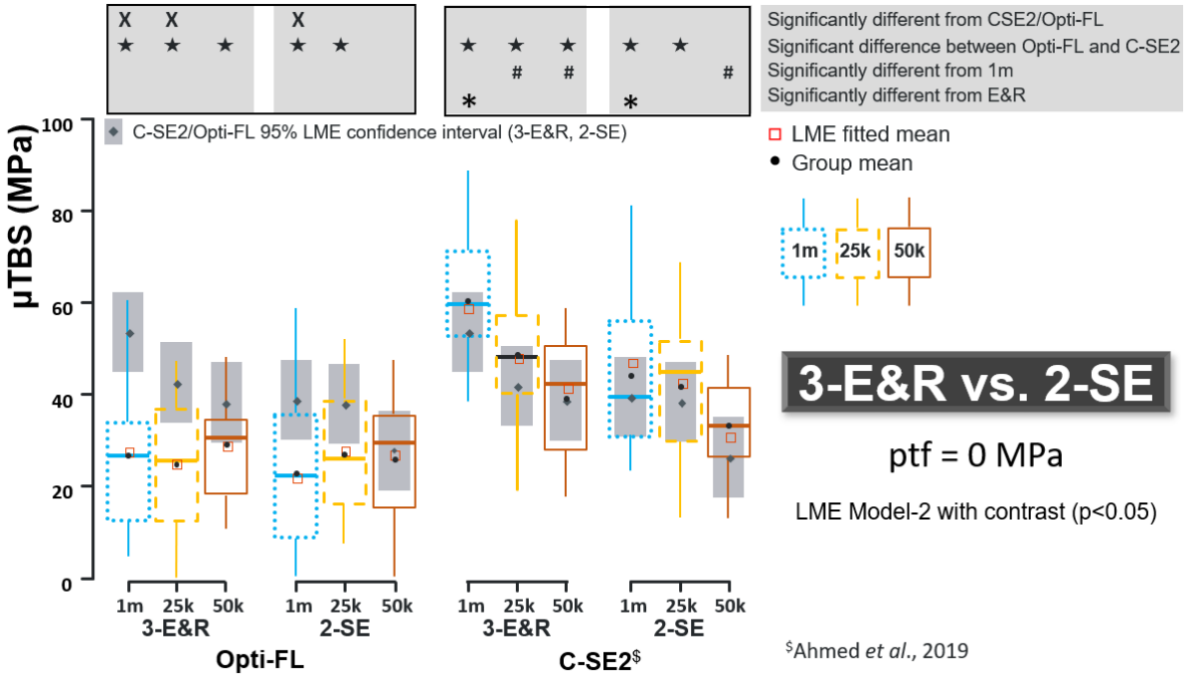
Significantly different from CSE2/Opti-FL
Significant difference between Opti-FL and C-SE2
Significantly different from 1m
Significantly different from E&R
C-SE2/Opti-FL 95% LME confidence interval (3-E&R, 2-SE)
LME fitted mean
Group mean
μTBS (MPa)
100
80
60
40
20
0
1m 25k 50k
3-E&R
2-SE
Opti-FL
C-SE2$
3-E&R vs. 2-SE
ptf = 0 MPa
LME Model-2 with contrast (p<0.05)
$Ahmed et al., 2019

FIG. 4A

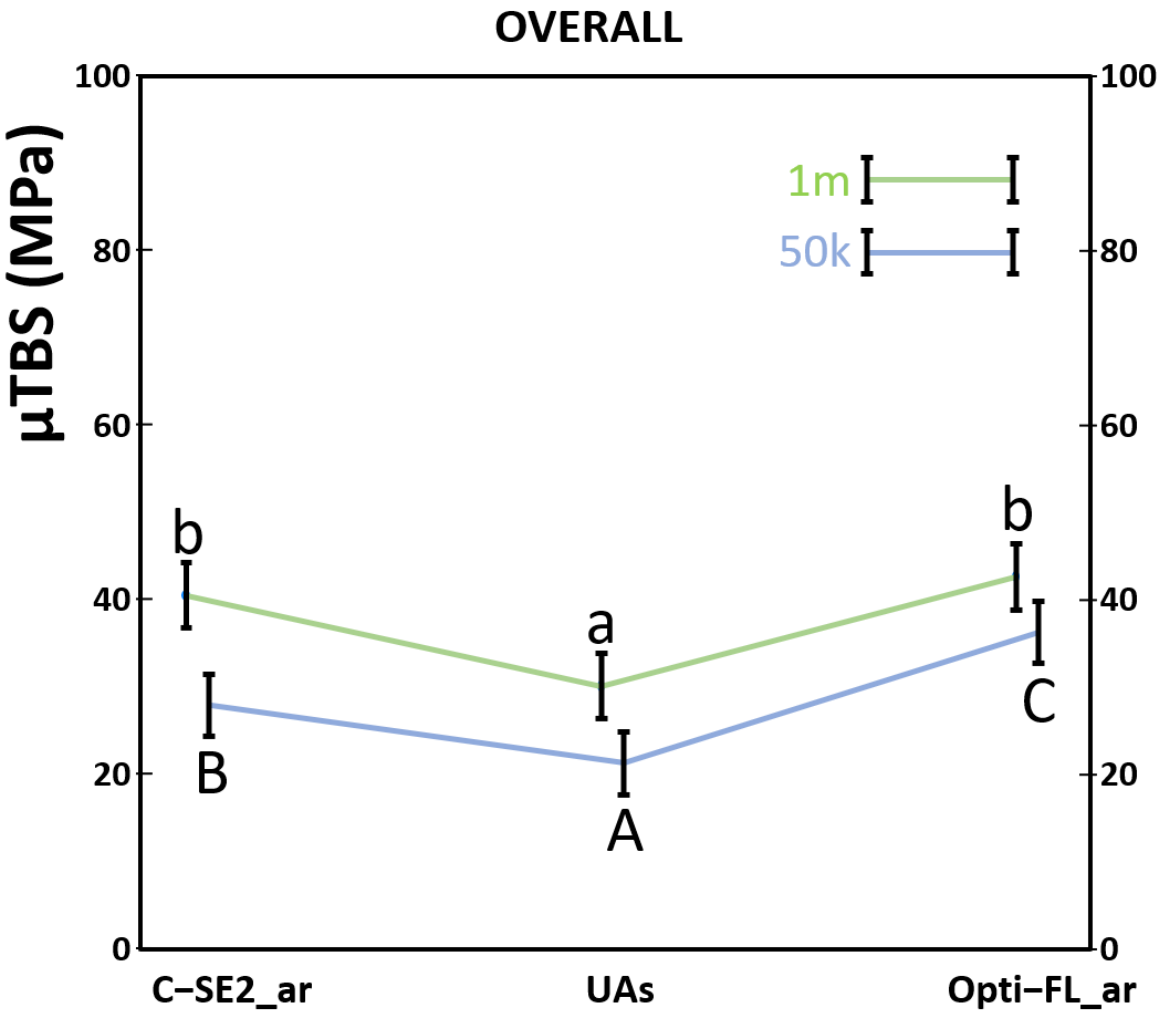
OVERALL
µTBS (MPa)
100
80
60
40
20
0
1m
50k
b
a
b
B
A
C
C–SE2_ar
UAs
Opti–FL_ar

FIG. 4B

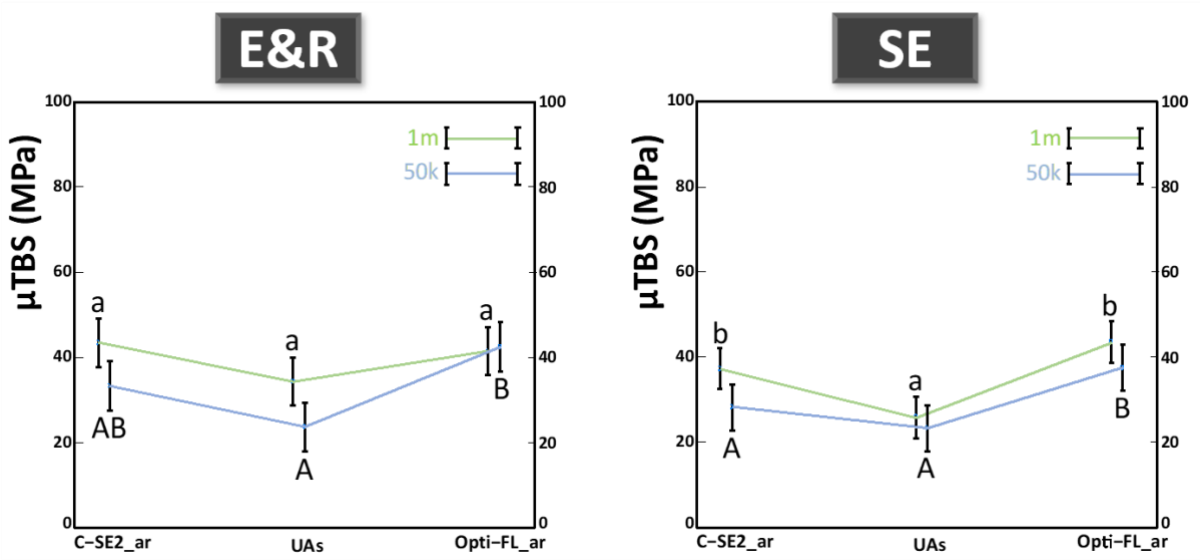
E&R
SE
µTBS (MPa)
1m
50k
a
AB
a
A
a
B
b
A
a
A
b
B
C–SE2_ar
UAs
Opti–FL_ar
0
20
40
60
80
100

FIG. 5

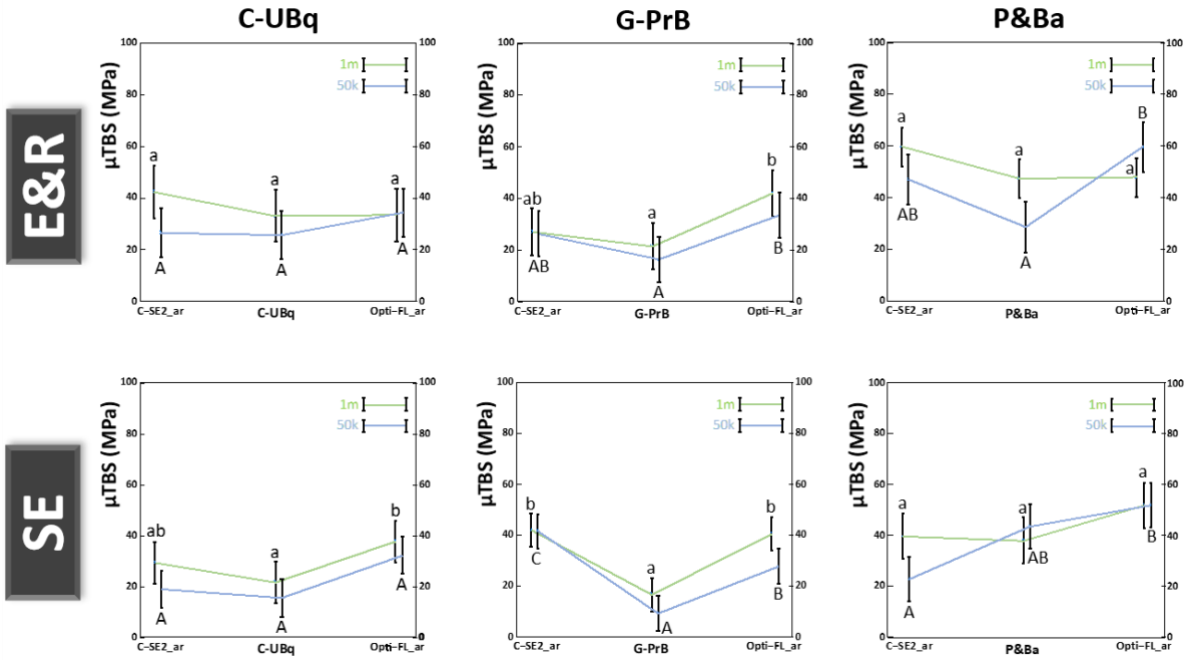
C-UBq
G-PrB
P&Ba
E&R
SE
µTBS (MPa)
1m
50k
C-SE2_ar
C-UBq
G-PrB
P&Ba
Opti-FL_ar

FIG. 6

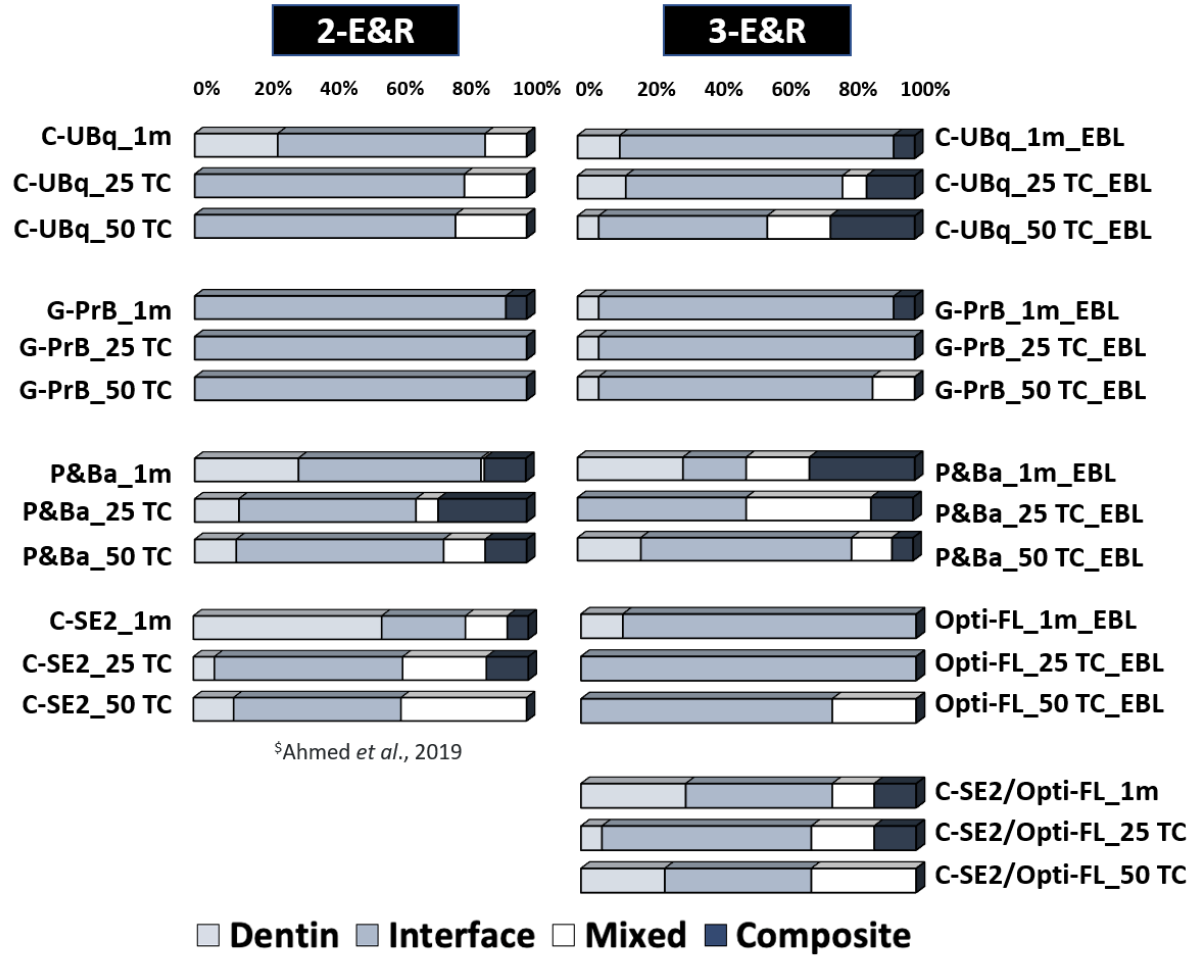
2-E&R
3-E&R
0%
20%
40%
60%
80%
100%
C-UBq_1m
C-UBq_25 TC
C-UBq_50 TC
G-PrB_1m
G-PrB_25 TC
G-PrB_50 TC
P&Ba_1m
P&Ba_25 TC
P&Ba_50 TC
C-SE2_1m
C-SE2_25 TC
C-SE2_50 TC
$Ahmed et al., 2019
C-UBq_1m_EBL
C-UBq_25 TC_EBL
C-UBq_50 TC_EBL
G-PrB_1m_EBL
G-PrB_25 TC_EBL
G-PrB_50 TC_EBL
P&Ba_1m_EBL
P&Ba_25 TC_EBL
P&Ba_50 TC_EBL
Opti-FL_1m_EBL
Opti-FL_25 TC_EBL
Opti-FL_50 TC_EBL
C-SE2/Opti-FL_1m
C-SE2/Opti-FL_25 TC
C-SE2/Opti-FL_50 TC
Dentin
Interface
Mixed
Composite

FIG. 7

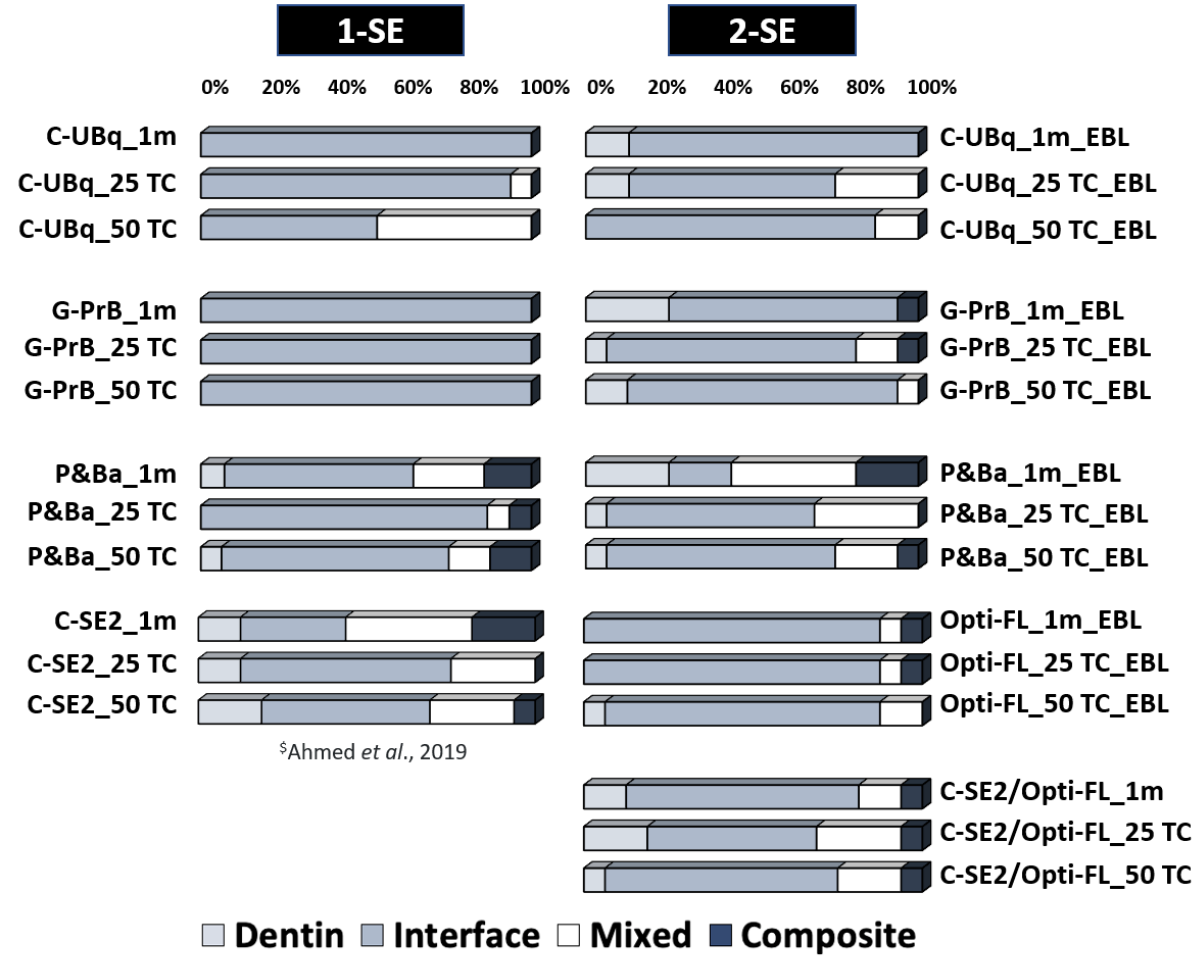
1-SE
2-SE
0% 20% 40% 60% 80% 100% 0% 20% 40% 60% 80% 100%
C-UBq_1m
C-UBq_25 TC
C-UBq_50 TC
C-UBq_1m_EBL
C-UBq_25 TC_EBL
C-UBq_50 TC_EBL
G-PrB_1m
G-PrB_25 TC
G-PrB_50 TC
G-PrB_1m_EBL
G-PrB_25 TC_EBL
G-PrB_50 TC_EBL
P&Ba_1m
P&Ba_25 TC
P&Ba_50 TC
P&Ba_1m_EBL
P&Ba_25 TC_EBL
P&Ba_50 TC_EBL
C-SE2_1m
C-SE2_25 TC
C-SE2_50 TC
Opti-FL_1m_EBL
Opti-FL_25 TC_EBL
Opti-FL_50 TC_EBL
$Ahmed et al., 2019
C-SE2/Opti-FL_1m
C-SE2/Opti-FL_25 TC
C-SE2/Opti-FL_50 TC
Dentin
Interface
Mixed
Composite

FIG. 8

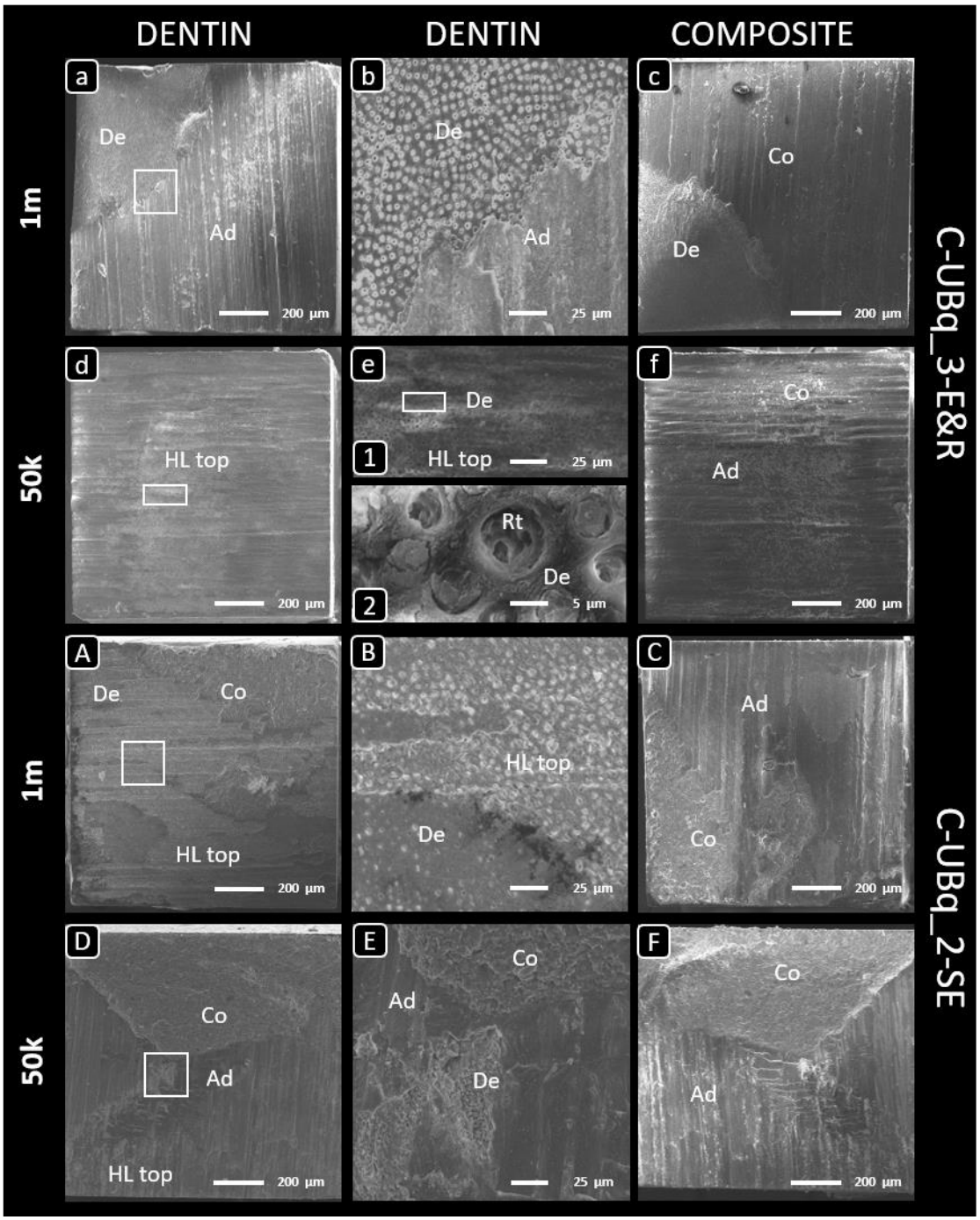
DENTIN
DENTIN
COMPOSITE
1m
50k
1m
50k
C-UBq_3-E&R
C-UBq_2-SE
a
b
c
d
e
f
A
B
C
D
E
F
1
2
De
Ad
Co
HL top
Rt
200 µm
25 µm
5 µm

FIG. 9

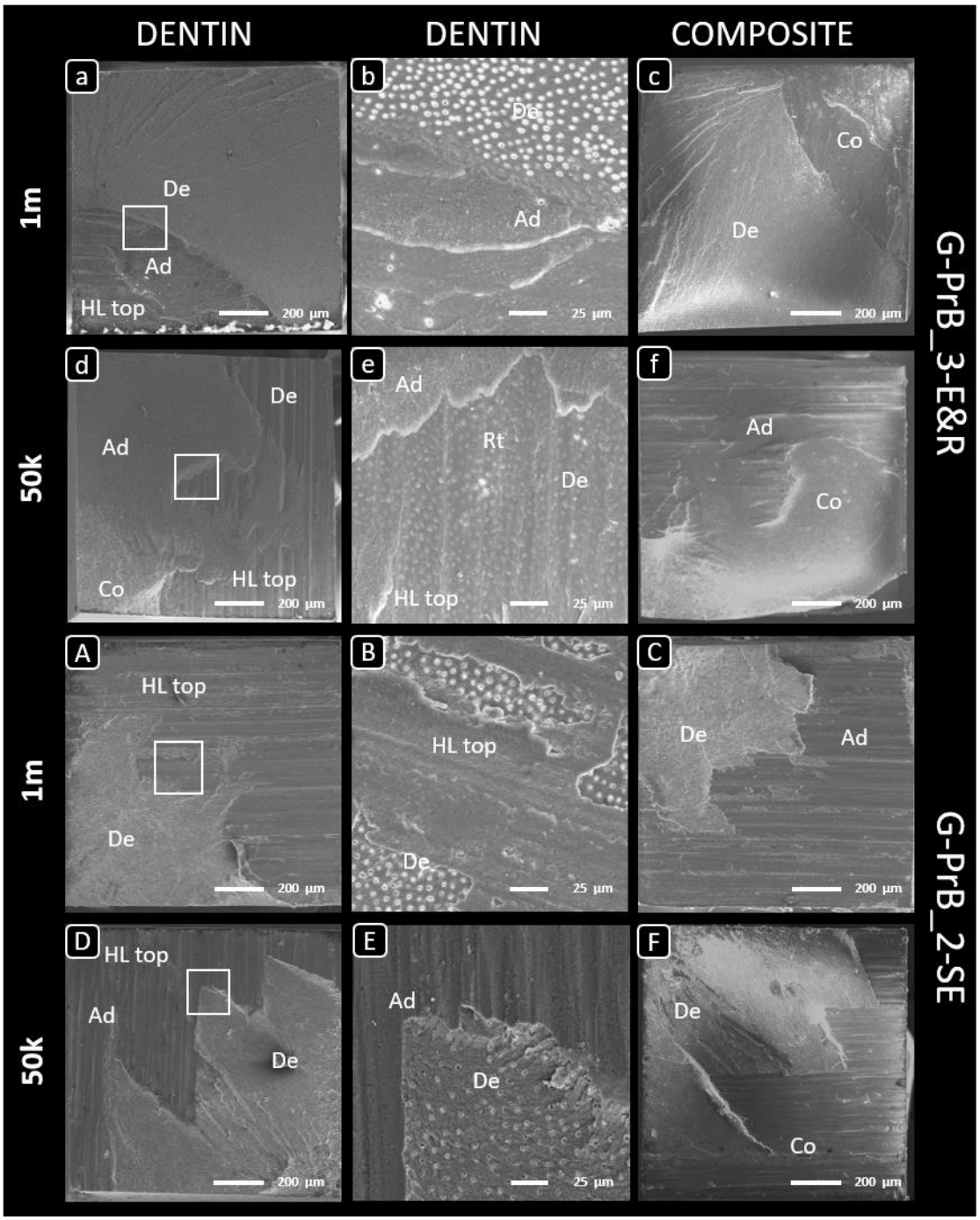
DENTIN
DENTIN
COMPOSITE
1m
50k
1m
50k
G-PrB_3-E&R
G-PrB_2-SE
a
b
c
d
e
f
A
B
C
D
E
F
De
Ad
HL top
Co
Rt
200 µm
25 µm

FIG. 10

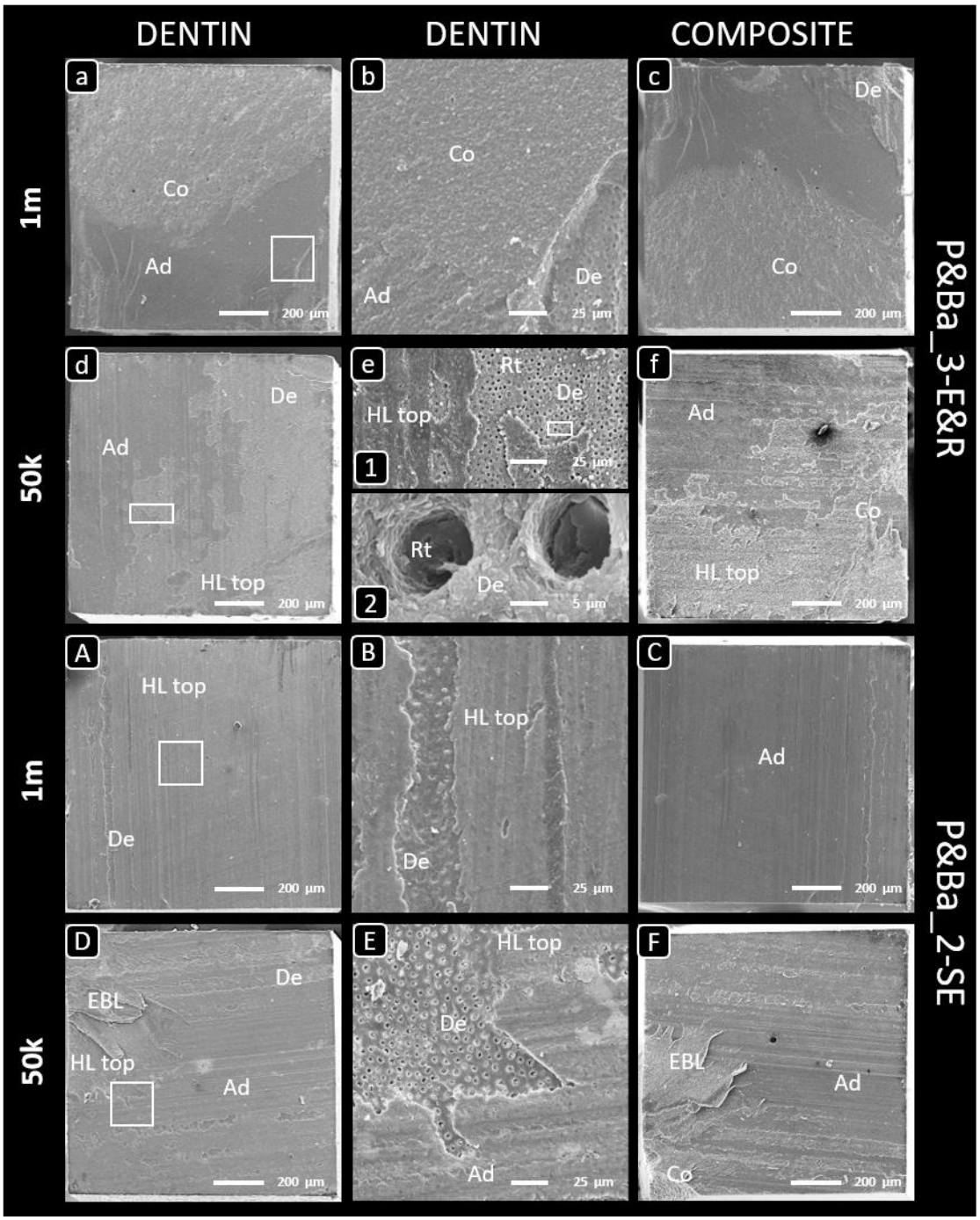
DENTIN
DENTIN
COMPOSITE
1m
50k
1m
50k
P&Ba_3-E&R
P&Ba_2-SE
a
b
c
d
e
f
A
B
C
D
E
F
Co
Ad
De
HL top
Rt
EBL
1
2
200 µm
25 µm
5 µm

FIG. 11

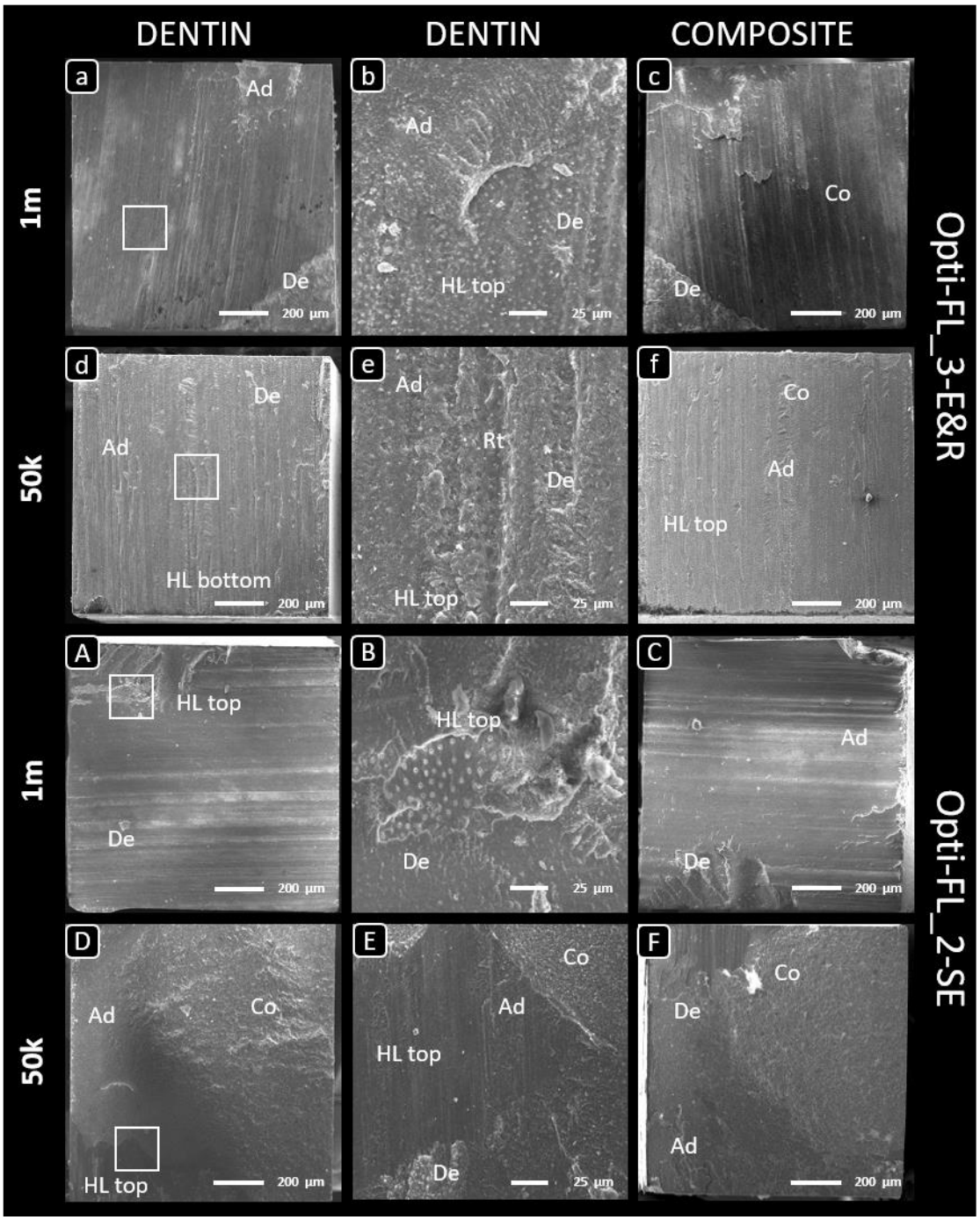
DENTIN
DENTIN
COMPOSITE
1m
50k
1m
50k
Opti-FL_3-E&R
Opti-FL_2-SE
a
Ad
De
200 µm
b
Ad
De
HL top
25 µm
c
Co
De
200 µm
d
De
Ad
HL bottom
200 µm
e
Ad
Rt
De
HL top
25 µm
f
Co
Ad
HL top
200 µm
A
HL top
De
200 µm
B
HL top
De
25 µm
C
Ad
De
200 µm
D
Ad
Co
HL top
200 µm
E
Co
Ad
HL top
De
25 µm
F
Co
De
Ad
200 µm

FIG. 12

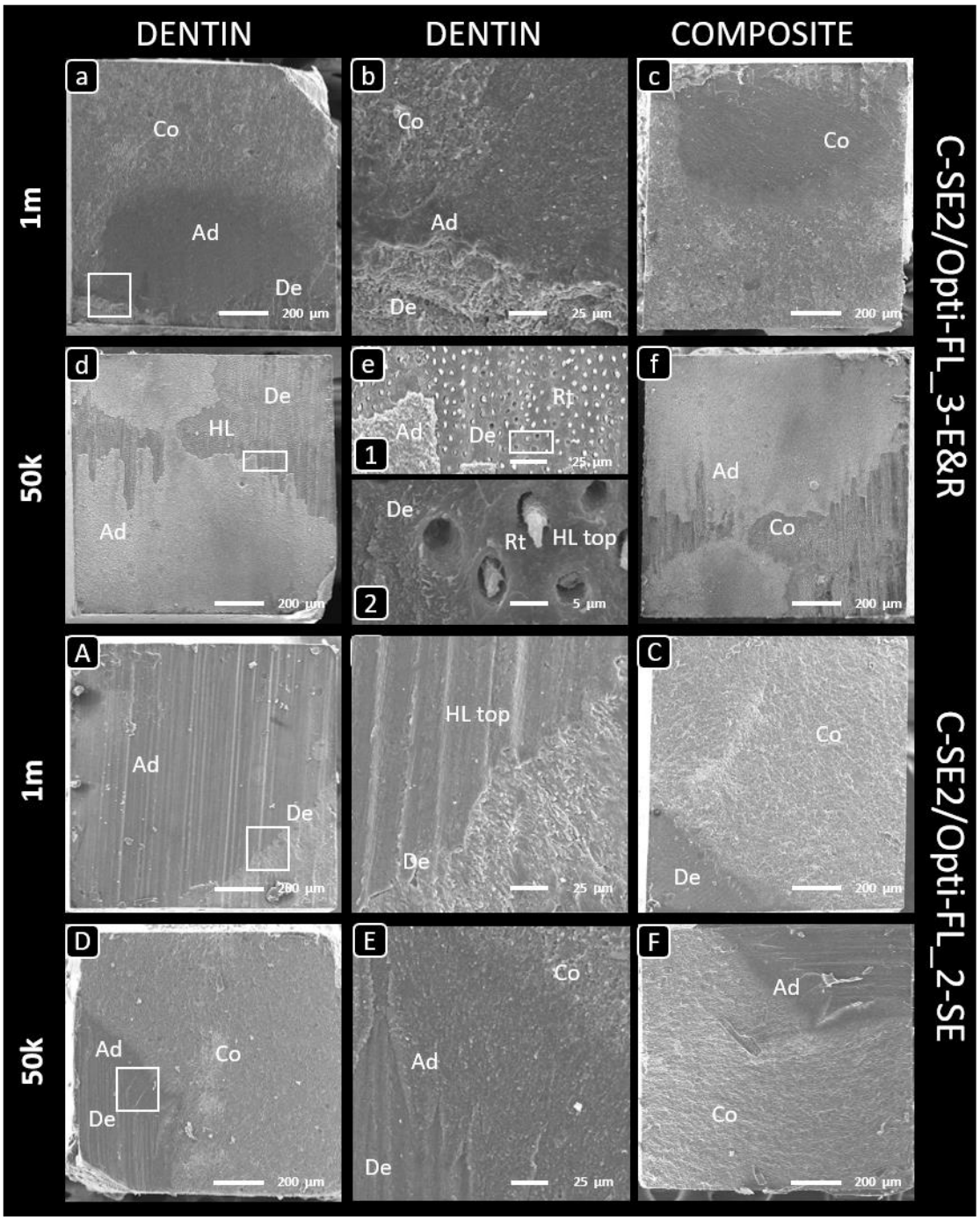
DENTIN
DENTIN
COMPOSITE
1m
50k
1m
50k
C-SE2/Opti-FL_3-E&R
C-SE2/Opti-FL_2-SE
a
b
c
d
e
f
A
C
D
E
F
1
2
Co
Ad
De
HL
HL top
Rt
200 µm
25 µm
5 µm

FIGURE LEGENDS

Figure 1. Split box-whisker plots (min-[lower quartile–median-upper quartile]-max) of μTBS (in MPa) of the universal adhesives (UAs) (Clearfil Universal Bond Quick, Kuraray Noritake: 'C-UBq'; G-Premio Bond, GC: 'G-PrB'; Prime&Bond Active, Dentsply Sirona: 'P&Ba') to dentin after 1-month water storage without thermocycling (blue color; 1m), upon additional 25k (orange color) and 50k TC (black color) and when the adhesives were applied in E&R mode. The '2-E&R' mode (continuous line; left half) represents the application of UA following the respective manufacturer's instructions (without EBL); the '3-E&R' mode (dotted line; right half) represents the application of UA as primer (not light-cured) followed by the extra bonding layer (EBL: OptiBond FL adhesive (Kerr)) that was light-cured. The black closed dots and the open red squares represent the mean μTBS and the fitted linear mixed-effects (LME) means, respectively. The thick horizontal line within each box represents the median. The light grey boxes in the background represent the 95% LME confidence interval for the reference adhesive OptiBond FL (Kerr; 'Opti-FL') applied in 3-E&R mode, with the diamond indicating the mean μTBS. Statistically significant differences between experimental groups according to LME Model-1 are indicated by specific symbols and explained in the graph legend at the top-right side.

Figure 2. Split box-whisker plots (min-[lower quartile–median-upper quartile]-max) of μTBS (in MPa) of the universal adhesives (UAs) (Clearfil Universal Bond Quick, Kuraray Noritake: 'C-UBq'; G-Premio Bond, GC: 'G-PrB'; Prime&Bond Active, Dentsply Sirona: 'P&Ba') to dentin after 1-month water storage without thermocycling (blue color; 1m), upon additional 25k (orange color) and 50k TC (black color) and when the adhesives were applied in SE mode. The '1-SE' mode (continuous line; left half) represents the application of UA following the respective manufacturer's instructions; the '2-SE' mode (dotted line; right half) represents the application of UA as primer (not light-cured) followed by the extra bonding layer (EBL: OptiBond FL adhesive (Kerr)) that was light-cured. The black closed dots and the open red squares represent the mean μTBS and the fitted linear mixed-effects (LME) means, respectively. The thick horizontal line within each box represents the median. The light grey boxes in the background represent the 95% LME confidence interval for the reference adhesive OptiBond FL (Kerr; 'Opti-FL') applied in an experimental 2-SE mode, with the diamond indicating the mean μTBS. Statistically significant differences between experimental groups according to LME Model-1 are indicated by specific symbols and explained in the graph legend at the top-right side.

Figure 3. Box-whisker plots (min-[lower quartile–median-upper quartile]-max) of μTBS (in MPa) of, respectively, the gold-standard E&R and SE reference adhesives OptiBond FL (Kerr: 'Opti-FL') and Clearfil SE Bond 2 (Kuraray Noritake: 'C-SE2') to dentin after 1-month water storage without thermocycling (dotted blue line; 1m), upon additional 25k (dashed yellow line) and 50k TC (continuous

orange line). The '3-E&R' mode represents the application of the reference 3-E&Ra Opti-FL following the respective manufacturer's instructions and the application of the reference 2-SEa C-SE2 following an experimental 3-E&R mode (not according to the manufacturer's instructions), and vice versa for the '2-SE' mode. The black closed dots and the open red squares represent the mean μTBS and the fitted linear mixed-effects (LME) means, respectively. The thick horizontal line within each box represents the median. The light grey boxes in the background represent the 95% LME confidence interval for the experimental cross-reference adhesive, combining Clearfil SE Bond 2 primer (Kuraray Noritake) with the OptiBond FL (Kerr) adhesive resin, referred to as 'C-SE2/Opti-FL' and applied in 3-E&R and 2-SE modes. The diamonds indicate the LME mean μTBS. Statistically significant differences between experimental groups according to LME Model-2 are indicated by specific symbols and explained in the graph legend at the top-right side.

Figure 4. Line graph presenting the overall μTBS (in MPa with 95% confidence intervals) of the universal adhesives (UAs: Clearfil Universal Bond Quick, Kuraray Noritake; G-Premio Bond, GC; Prime&Bond Active, Dentsply Sirona) combined, when bonded to dentin following the respective manufacturer's instructions (middle), and as primer (not light-cured) followed by the extra bonding layer (EBL) Clearfil SE Bond 2 adhesive resin (C-SE2_ar; Kuraray Noritake) (left) and OptiBond FL adhesive resin (Opti-FL_ar; Kerr) (right), with solely EBL light-cured (E&R and SE mode combined) (a), and these when applied in E&R and SE mode after 1-month water storage without thermocycling (1m) and upon additional 50k TC (b). Statistically significant differences in overall μTBS according to LME Model-3 are indicated by different small and capital letters for 1m and 50k, respectively.

Figure 5. Line graphs presenting the μTBS (in MPa with 95% confidence intervals) of the universal adhesives (UAs) Clearfil Universal Bond Quick, Kuraray Noritake (C-UBq), G-Premio Bond, GC (G-PrB), and Prime&Bond Active, Dentsply Sirona (P&Ba) individually, when in each graph bonded to dentin following the respective manufacturer's instructions (middle), and as primer (not light-cured) followed by the extra bonding layer (EBL) Clearfil SE Bond 2 adhesive resin (C-SE2_ar; Kuraray Noritake) (left) and OptiBond FL adhesive resin (Opti-FL_ar; Kerr) (right), with solely EBL light-cured, and these when applied in E&R and SE mode after 1-month water storage without thermocycling (1m) and upon additional 50k TC. Statistically significant differences in μTBS according to LME Model-3 are indicated by different small and capital letters for 1m and 50k, respectively.

Figure 6. Light-microscopy failure analysis for the UAs applied in a 2-E&R (without EBL$^{(1)}$) and 3-E&R (with EBL) bonding mode, and for the cross-reference adhesive C-SE2/Opti-FL (bottom) applied in a 3-E&R mode.

Figure 7. Light-microscopy failure analysis for the UAs applied in a 1-SE (without EBL[(1)]) and 2-SE (with EBL) bonding mode, and for the cross-reference adhesive C-SE2/Opti-FL (bottom) applied in a 2-SE mode.

Figure 8. SEM failure analysis of C-UBq_EBL applied in 3-E&R (a-f) and 2-SE (A-F) bonding mode at 1 month (1m) without TC (a-c; A-C) and upon additional 50k TC (d-f; D-F). **(a-c: C-UBq_3-E&R_1m)** Representative SEM photomicrographs of a 'mixed failure' at the dentin side in (a), with high magnification of the white square in (b), and at the composite side in (c). **(d-f: C-UBq_3-E&R_50k TC)** Representative SEM photomicrographs of an 'interfacial failure' at the dentin side in (d), with high magnification of the white rectangular in (e1), ultra-high magnification of the white rectangular in (e2), and at the composite side in (f). **(A-C: C-UBq_2-SE_1m)** Representative SEM photomicrographs of a 'mixed failure' at the dentin side in (A), with high magnification of the white square in (B), and at the composite side in (C). **(D-F: C-UBq_2-SE_50k TC)** Representative SEM photomicrographs of a 'mixed failure' at the dentin side in (D), with high magnification of the white rectangle in (E), indicating partial attachment of the adhesive layer and composite to dentin, and at the composite side in (F). Ad: Adhesive resin; Co: Composite resin; De: Dentin; HL: Hybrid layer; Rt: Resin tag.

Figure 9. SEM failure analysis of G-PrB_EBL applied in 3-E&R (a-f) and 2-SE (A-F) mode at 1 month (1m) without TC (a-c; A-C) and upon additional 50k TC (d-f; D-F). **(a-c: G-PrB_3-E&R_1m)** Representative SEM photomicrographs of a 'mixed failure' at the dentin side in (a), with high magnification of the white square in (b), showing the bottom of the hybrid layer as well as the adhesive layer, and at the composite side in (c). **(d-f: G-PrB_3-E&R_50k TC)** Representative SEM photomicrographs of a 'mixed failure' at the dentin side in (d), with high magnification of the white square in (e), showing both fractured adhesive and the bottom of the hybrid layer, and at the composite side in (f), with the adhesive partially attached. **(A-C: G-PrB_2-SE_1m)** Representative SEM photomicrographs of a 'mixed failure' at the dentin side in (A), with high magnification of the white square in (B), showing dentin and the top of the hybrid layer, and at the composite side in (C), with dentin partially attached. **(D-F: G-PrB_2-SE_50k TC)** Representative SEM photomicrographs of a 'mixed failure' at the dentin side in (D), with high magnification of the white rectangle in (E), showing fractured dentin and a partially attached adhesive layer, and at the composite side in (F), with fractured dentin partially attached. Ad: Adhesive resin; Co: Composite resin; De: Dentin; HL: Hybrid layer; Rt: Resin tag.

Figure 10. SEM failure analysis of P&Ba_EBL applied in 3-E&R (a-f) and 2-SE (A-F) mode at 1 month (1m) without TC (a-c; A-C) and upon additional 50k TC (d-f; D-F). **(a-c: P&Ba_3-E&R_1m)**

Representative SEM photomicrographs of a 'mixed failure' at the dentin side in (a), with high magnification of the white square in (b), showing composite and fractured dentin, and at the composite side in (c), showing partially attached fractured dentin. **(d-f: P&Ba_3-E&R_50k TC)** Representative SEM photomicrographs of an 'interfacial failure' at the dentin side in (d), with high magnification of the white rectangular in (e1), showing the top of the hybrid layer and dentinal tubules, and ultra-high magnification of the white rectangular in (e2), showing fractured resin tags within the dentinal tubules, and at the composite side in (f), with a partially attached fractured adhesive layer. **(A-C: P&Ba_2-SE_1m)** Representative SEM photomicrographs of an 'interfacial failure' at the dentin side in (A), with high magnification of the white square in (B), showing the top of the hybrid layer, and at the composite side in (C). **(D-F: P&Ba_2-SE_50k TC)** Representative SEM photomicrographs of an 'interfacial failure' at the dentin side in (D), showing a cohesive failure between adhesive and EBL, with high magnification of the white rectangle in (E), showing the top and bottom of the hybrid layer as well as dentin, and at the composite side in (F), showing a partially attached adhesive and EBL layer. Ad: Adhesive resin; Co: Composite resin; De: Dentin; EBL: Extra bonding layer; HL: Hybrid layer; Rt: Resin tag.

Figure 11. SEM failure analysis of Opti-FL applied in 3-E&R (a-f) and 2-SE (A-F) mode at 1 month (1m) without TC (a-c; A-C) and upon additional 50k TC (d-f; D-F). **(a-c: Opti-FL_3-E&R_1m)** Representative SEM photomicrographs of an 'interfacial failure' at the dentin side in (a), showing fractured dentin, with high magnification of the white square in (b), showing the top of the hybrid layer and a fractured adhesive layer, and at the composite side in (c), showing partially fractured dentin. **(d-f: Opti-FL_3-E&R_50k TC)** Representative SEM photomicrographs of an 'interfacial failure' at the dentin side in (d), with high magnification of the white square in (e), showing the bottom of the hybrid layer and resin tags, and at the composite side in (f), showing a partially fractured adhesive layer with the top of the hybrid layer. **(A-C: Opti-FL_2-SE_1m)** Representative SEM photomicrographs of an 'interfacial failure' at the dentin side in (A), showing the top of the hybrid layer and fractured dentin, with high magnification of the white square in (B), showing the hybrid layer, and at the composite side in (C) showing partially fractured dentin and the adhesive layer **(D-F:Opti-FL_2-SE_50k TC)** Representative SEM photomicrographs of a 'mixed failure' at the dentin side in (D), showing partially fractured composite, with high magnification of the white rectangle in (E), showing dentin, top of the hybrid layer, the adhesive and partially fractured composite, and at the composite side in (F), showing partially fractured dentin. Ad: Adhesive resin; Co: Composite resin; De: Dentin; HL: Hybrid layer; Rt: Resin tag.

Figure 12. SEM failure analysis of CSE2/Opti-FL applied in 3-E&R (a-f) and 2-SE (A-F) mode at 1 month (1m) without TC (a-c; A-C) and upon additional 50k TC (d-f; D-F). **(a-c: CSE2/Opti-FL_3-E&R_1m)** Representative SEM photomicrographs of a 'mixed failure' at the dentin side in (a), showing fractured composite, with high magnification of the white square in (b), showing the adhesive, composite and dentin, and at the composite side in (c). **(d-f: CSE2/Opti-FL_3-E&R_50k TC)** Representative SEM photomicrographs of an 'interfacial failure' at the dentin side in (d), with high magnification of the white rectangular in (e1), showing the bottom of the hybrid layer and a partially attached adhesive layer, ultra-high magnification of the white rectangular in (e2), showing fractured resin tags and the top of the hybrid layer, and at the composite side in (f). **(A-C: CSE2/Opti-FL _2-SE_1m)** Representative SEM photomicrographs of a 'mixed failure' at the dentin side in (A), with high magnification of the white square in (B), and at the composite side in (C), showing partially fractured dentin attached. **(D-F: CSE2-FL_2-SE_50k TC)** Representative SEM photomicrographs of a 'mixed failure' at the dentin side in (D), with high magnification of the white square in (E), and at the composite side in (F). Ad: Adhesive resin; Co: Composite resin; De: Dentin; HL: Hybrid layer; Rt: Resin tag.