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Shear Bond Strength of Adhesives Placed following Selective Removal of Red-Fluorescing Carious Dentine in vitro

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Keywords
Dentine bonding agent · Dental caries · Self-etch · Total etch · Fracture mode

Abstract
Red-fluorescing dentine indicates bacterial contamination [Caries Res 2002; 36: 315–319]. We investigated effect of removal of red fluorescent dentine caries on shear bond strength and fracture mode of 4 adhesive approaches. Sixty-five carious teeth and 50 noncarious controls were distributed into 4 groups: Clearfil™ self-etch (CSE), OptiBond™ FL total etch (OTE), Scotchbond™ Universal total etch (STE) and self-etch (SSE). Samples were excited at 405 nm and viewed through 530 nm filter. Carious samples were ground flat exposing strongly red-fluorescing (StrongRF) dentine, on which a composite cylinder was placed, using one of 4 adhesives. After 22 h in water, shear bond strength and fracture mode were analysed. StrongRF was removed; composite cylinders were placed on weakly red-fluorescing (WeakRF) dentine and tested as described above. Finally, red-fluorescing dentine was removed, and composite cylinders were placed on non-fluorescing (NonRF) dentine and tested. Composites were placed at 3 corresponding heights in controls. After 22 h in water, shear bond strength testing and fracture mode analysis were performed. Differences were tested using Mann-Whitney or Wilcoxon tests ($p \leq 0.05$). Median (Q1, Q3) shear bond strength on StrongRF was SSE 14.4 (9.2, 18.2) MPa >CSE 10.2 (6.4, 17.3) MPa >STE 9.1 (6.9, 11.2) MPa >OTE 6.8 (4.0, 10.8) MPa. Shear bond strength increased statistically significantly for all adhesives on WeakRF: SSE 19.8 (13.6, 24.3) MPa >STE 19.5 (12.7, 23.1) MPa >CSE 17.5 (12.0, 22.5) MPa >OTE 15.8 (11.9, 20.9) MPa. Only STE 25.6 (22.4, 29.1) MPa and CSE 22.1 (17.6, 24.6) MPa were significantly different on NonRF compared to WeakRF. For controls tested at corresponding depths, superficial shear bond strength was OTE 18.7 (16.0, 22.1) MPa >STE 18.4 (12.0, 25.9) MPa >CSE 18.1 (12.7, 20.7) MPa >SSE 13.0 (9.6, 17.8) MPa. This was significantly higher compared to StrongRF except for SSE. Central shear bond strength was not significantly different to WeakRF, deep shear bond strength was significantly lower for SSE and CSE but higher for OTE compared to carious. Conclusion: StrongRF dentine should be removed for higher shear bond strength, but WeakRF dentine can often be preserved without compromising adhesive bond strength.

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Introduction

Although early-stage caries can be treated non-invasively, once a cavity develops that cannot be kept clean by the patient, an undisturbed biofilm forms, and a microbiota shift in favour of more acidogenic species occurs [Lennon et al., 2002]. Under these conditions, caries can quickly spread deeper into the tooth. Restoration is often required to stop the progression of the lesion by cutting the supply of nutrients to any remaining microorganisms in the tooth and restoring a surface that the patient can keep clean [Fejerskov et al., 2015; Zandona and Longbottom, 2019; Machiulskiene et al., 2020]. Another reason for removing carious tissue is the need to provide a large enough surface area to allow restoration adhesion, inferring that inadequate caries removal may result in inferior bond strength and less durable restorations in the long term [Schwendicke et al., 2016].

The discussion about what exactly should be removed and how much tissue can be saved without compromising the longevity of the restoration has evolved towards a minimally invasive approach with the primary aim of preserving pulp vitality [Bjørndal et al., 1997; Kidd, 2004; Scholz et al., 2020; Widbiller et al., 2022]. Complete removal of carious tissue or non-selective caries removal is no longer recommended due to the increased risk of pulp exposure and unnecessary weakening of the tooth [Duncan et al., 2019]. Selective caries removal has been defined as the removal of carious dentine to one of two endpoints with either firm or soft dentine remaining [Innes et al., 2016].

Fusayama described two histologically different layers in carious dentine: the outer layer of infected dentine which is highly demineralized and with irreversible collagen breakdown and not capable of remineralization, and the inner layer of caries-affected dentine which is remineralizable and should be preserved [Fusayama, 1979]. Removal of the "heavily diseased portion" of carious dentine is still advised, whereas caries-affected tissue can be sealed in under the restoration [Meyer-Lueckel and Paris, 2016]. It is difficult for the clinician to differentiate between the infected or contaminated layer and the caries-affected dentine using traditional tactile methods [Manton, 2013; Innes et al., 2016].

Several methods have been suggested that support selective caries removal by helping to differentiate between dentine that should be removed and that which should be conserved, but some have been shown to result in overexcavation [Lager et al., 2003]. Chemo-mechanical processes use NaOH to partially dissolve collagen in order to then remove softened dentine. Polymer instruments are characterized by an adjusted hardness allowing them to selectively remove softened carious dentine and leaving caries-affected dentine behind [Ferraz et al., 2015].

Red autofluorescence in carious dentine has been shown to be a reliable and easily used marker for infected portions of the lesion [Lennon et al., 2002; Trippe et al., 2020]. Fluorescence-aided caries excavation (FACE) may be used to *selectively* remove heavily infected dentine without resulting in overexcavation [Lennon et al., 2007; Zhang et al., 2013]. It is not yet clear whether this remaining tissue is suitable as a foundation for restorations and which adhesive may be used to produce a high-quality bond to this surface.

Following a selective approach, where red-fluorescing dentine is removed and dentine which does not fluoresce red is preserved, a large proportion of the cavity floor available for bonding restorations will consist of caries-affected dentine which is histologically different to sound dentine. The bond strength of caries-affected dentine has been shown to be lower than to normal dentine [Nakajima et al., 2011]. Although microhardness and mineral density in dentine following FACE have been reported [Zhang et al., 2013; Lai et al., 2014], the effect of FACE on bond strength of subsequent restorations has not been widely investigated. Therefore, the objective of this study was to investigate the effect of selective removal of red fluorescent dentine caries at three different depths on shear bond strength and fracture mode using four different adhesive approaches.

Materials and Methods

Extracted human permanent teeth with primary carious lesions and matching noncarious controls were used for the investigation. The teeth were collected in light-proof containers and stored for between 6 weeks and 6 months at 4°C in 0.5% chloramine T solution (Merck KGaA, Darmstadt, Germany) until use. The remaining soft tissue and calculus were removed using a scaler (Hu-Friedy ST2/37, Chicago, IL, USA). A radiograph of each tooth was taken (Heliodent, Sirona, Bensheim, Germany) and independently scored by 2 examiners (NSR and ÁML) using the ICDAS radiographic scoring system [Pitts et al., 2014]. In cases of disagreement, the decision was reached by consensus. Teeth scoring 0 (sound) or with dentine caries (RA3–RC5) were selected. Samples with caries only into enamel or lesions extending into the pulp were excluded. Sixty-five carious teeth were stratified into 4 groups according to lesion size, tooth type, and lesion localization. Each of the 4 groups was assigned to a different dental adhesive approach.

- Scotchbond Universal total etch (STE) mode (3M ESPE, Seefeld, Germany).
- Scotchbond Universal self-etch (SSE) mode (3M ESPE, Seefeld, Germany).
- Clearfil self-etch (CSE) (Kuraray, Tokyo, Japan).

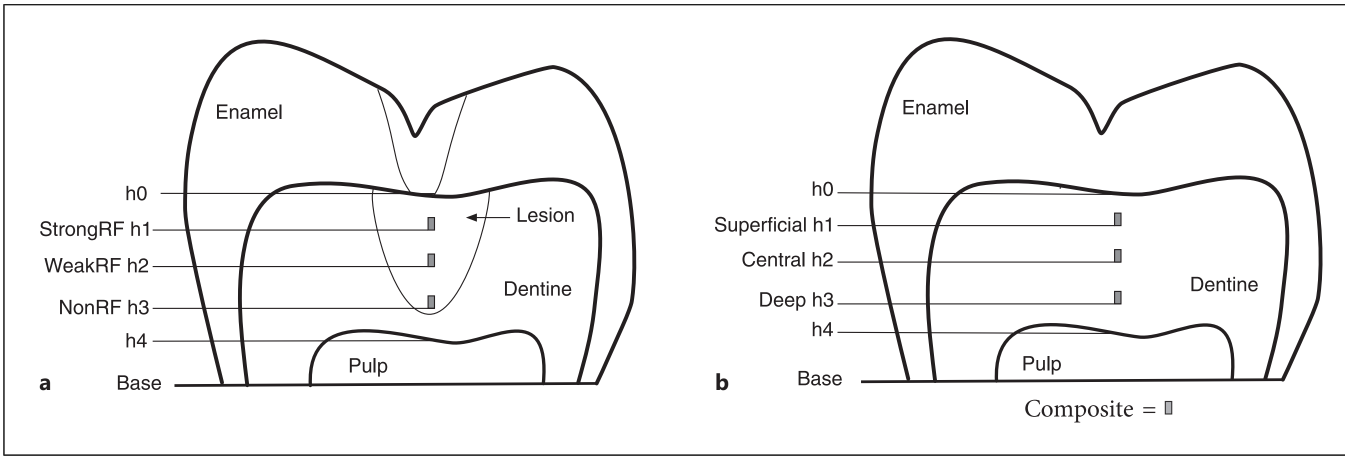

Fig. 1. Diagram (not to scale) showing position of composite restorations in carious (**a**) and noncarious (**b**) samples at heights h1, h2, and h3.

- OptiBond FL total etch (OTE) (Kerr, Orange, USA).

Fifty sound teeth (noncarious controls) were stratified according to tooth type and evenly assigned to each of the 4 groups as controls.

Sample Preparation

Soft, wet, disorganized carious tissue was first removed from the surface of the lesion in carious samples using a hand excavator without any pressure (Kerr Dental, Brea, CA, USA). After removing the roots, the sample teeth were embedded in a cylinder of chemically cured acrylic resin (SamplKwick, Buehler, Lake Bluff, IL, USA) with the carious lesion at the centre of the sample body, visible on the sample surface, and mounted on a single black 2 × 3 LEGO® brick (3002 LEGO, Billund, Denmark) to allow accurate repositioning for all subsequent photographs of the same sample. Graphite powder 423 (West System, Bay City, USA) was added to the polymer in a ratio of 1:50 per weight to suppress fluorescence from the acrylic. The samples were stored in tap water during and after preparation, and care was taken to ensure that the samples did not dry out during the experiment. Noncarious samples were prepared and mounted in the same way, although no lesion was present.

The enamel surface of all samples was ground down parallel to the sample base using Si-Carbide paper (FEPA P400 Buehler, Lake Bluff, IL, USA) and water to produce a flat surface having a diameter of at least 2 mm and removing most of the enamel from this aspect of the sample. A baseline height for each sample (h0) was recorded as the distance between the surface and the base of the sample body; see Figure 1a, b. This allowed the distances for h1–h4 to baseline to be recorded and for the distance from the pulp at h1, h2, and h3 to be calculated retrospectively.

Carious samples were ground down further to h1 as follows: The criteria for arriving at h1 in carious samples were firstly to remove any dentine which had been exposed to the surface in the case of cavitation and secondly to create a flat surface just into carious dentine with a diameter of at least 1.5 mm (h1; see Fig. 1, strongly red-fluorescing [StrongRF]). Thirdly, this dentine exhibited strong red fluorescence when excited at 405-nm excitation and viewed through a 530-nm emission filter (SiroInspect, Dentsply Sirona, Bensheim, Germany); see Figure 2. The visual assessment of fluorescent appearance was carried out by one examiner (NSR). All carious samples exhibited strong red fluorescence at h1.

In the noncarious samples, a similar height reduction was carried out in the absence of a lesion. The second height measurement (h1) distance between the surface and the base of the sample body was recorded; see Figure 1b.

Fluorescence Photography

Fluorescence images of the ground lesion surface of each sample at h1, h2, and h3 were made. Images were taken using a Canon EOS 550D camera (QLF-D Biluminator™ 2, Inspektor Research Systems BV, Bussum, the Netherlands) equipped with a 60-mm macro lens mounted on a camera stand. Images were recorded at 2,592 × 1,728 pixel resolution in RGB colour space. Fluorescence photographs were taken using the blue light setting (excitation light 405 nm) at a shutter speed of 1/20 s, aperture 8.0, custom white balance setting (calibrated in advance), and ISO 800. The fluorescence emission filter in the QLF-D Biluminator was replaced with a 530-nm emission filter (SiroInspect, Dentsply Sirona, Bensheim, Germany). This was done to allow photography under the same conditions used for FACE [Lennon et al., 2007]. The dentine surface in the superficial carious lesions was StrongRF (Fig. 2). In the noncarious samples, only green fluorescence was recorded.

Restoration Placement on StrongRF/Superficial Dentine at h1

In carious samples, restorations were placed at the centre of the lesion. To record the position of the restoration on the dentine surface at h1, 4 marks were placed in enamel on the sides of the samples (mesial, distal, oral, and lingual), which aligned with the location of the composite on the flattened dentine surface of the sample. These marks were later used to ensure that restorations placed at h2 and h3 would be at the same position, directly below the h1 restoration.

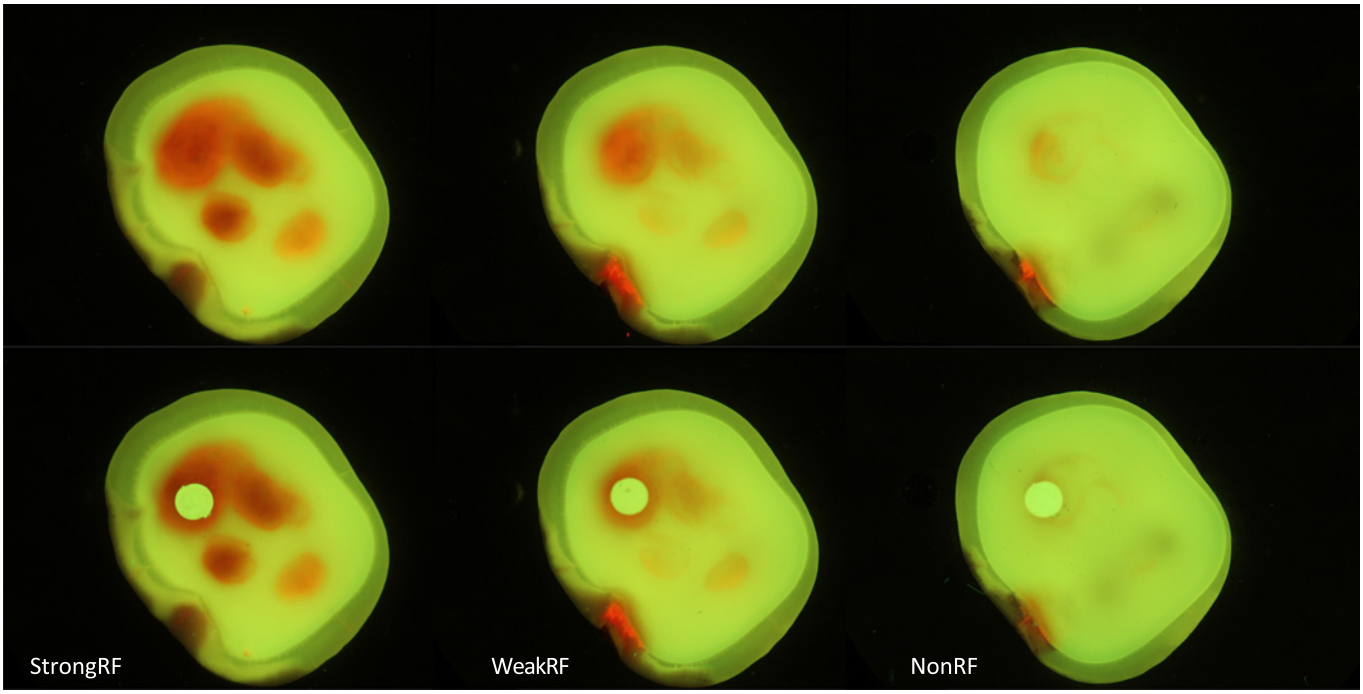

Fig. 2. Fluorescence images of a carious sample excavated to StrongRF, WeakRF, and NonRF top left to right. Bottom row: the same samples with a composite restoration placed on lesion area.

A custom-made precision polytetrafluoroethylene split mould (3 mm high, 16 mm diameter) with a central cylindrical cavity of 1.5 mm diameter was placed over the lesion on the prepared surface of the sample bodies using markings to centre and held in place by a custom-made holding device. The following dental adhesives were applied through the mouth of the Teflon mould according to the manufacturer's instructions: in the STE group, samples were treated with Scotchbond Universal (3M ESPE, Seefeld, Germany) in total etch mode (37% phosphoric acid; Ivoclar Vivadent, Schaan, Liechtenstein). Samples in the SSE group were also treated with Scotchbond Universal but in self-etch mode. Clearfil SE (Kuraray, Tokyo, Japan) was applied to the CSE group. In the OTE group, OptiBond FL was applied after etching with OptiBond FL Gel Etchant, 37.5% phosphoric acid (Kerr, Orange, CA, USA). Tetric Evo Ceram composite (Colour A3; Ivoclar Vivadent, Schaan, Liechtenstein) was placed in the cylindrical cavity to a height of 1.5 mm and light cured for 20 s (Bluephase C8; Ivoclar Vivadent, Schaan, Liechtenstein). The mould was detached, excess adhesive was removed with a scalpel blade, and the sample body was stored for 22 h in tap water at 37°C.

Shear Bond Strength

Shear bond strength for the restorations was measured in Newton using a universal testing machine (Zwick-0010, testXpert II V 2.1.; Zwick Roell, Ulm, Germany) at a cross head speed of 1 mm/min. The samples were positioned in the device so that the shearing stamp loaded the composite cylinder at an angle of 90° 200 µm from the tooth-adhesive interface until failure. Shear bond strength in MPa was calculated by dividing the peak force at failure (in *N*) by the bonding area (in mm^2). The effective bonding area calculated was 1.76 mm^2. The composite cylinders were collected for later fracture mode analysis in a scanning electron microscope (SEM).

Restoration Placement on Weakly Fluorescing/Central Dentine at h2

Carious samples were ground down further, removing the StrongRF portion of the lesion and exposing weakly red-fluorescing (WeakRF) dentine with a more orange-red appearance (Fig. 2). Red fluorescence in central carious lesions was less intense and mostly smaller in extension than in lesions seen at the superficial level. In noncarious samples, a similar height reduction was carried out in the absence of a lesion. The third height measurement (h2) distance between the surface and the base of the sample body was recorded (Fig. 1). The same procedure was followed for both carious and noncarious samples for height measurement, fluorescence photography, restoration placement, and shear bond strength testing as described for Strong RF/ superficial above.

Restoration Placement on Non-Fluorescing/Deep Dentine at h3

The samples were ground down further. In carious samples, WeakRF dentine was removed to a point where no more than a hint of yellow/orange fluorescence remained on the ground surface (Fig. 2, non-fluorescing [NonRF]). The fourth height measurement (h3) distance between the surface and the base of the sample body was recorded. Fluorescence images were taken as described above. A composite cylinder was placed and loaded until failure in the same way as described for StrongRF/superficial and WeakRF/ central.

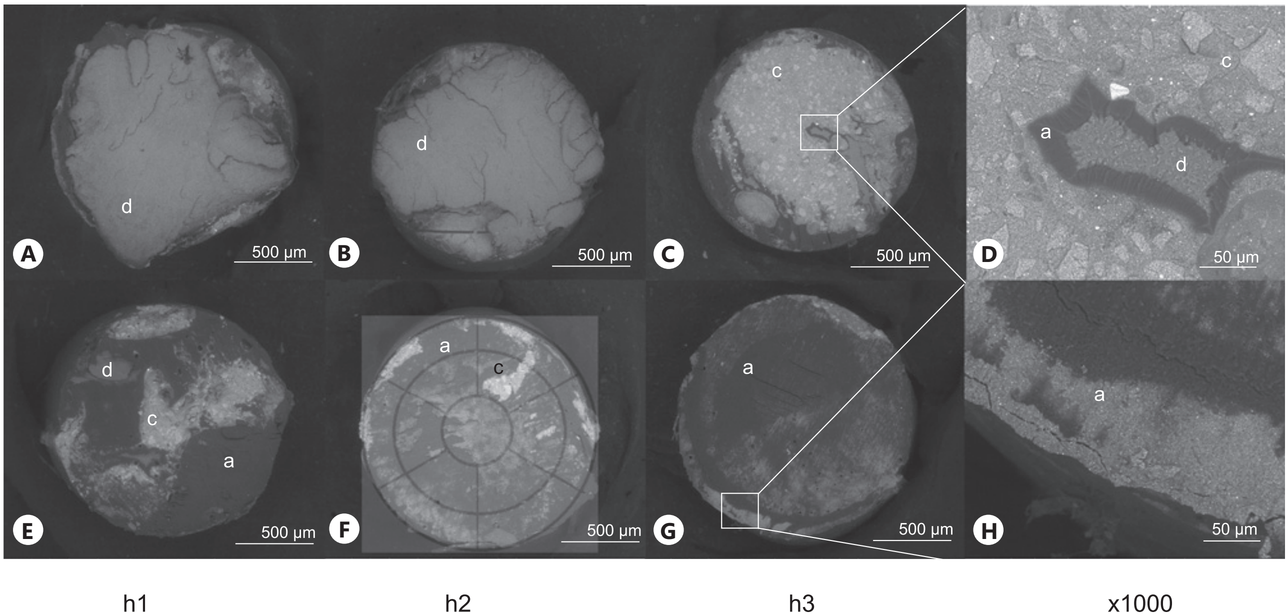

Fig. 3. Exemplary SEM images for fracture mode analysis. **A**–**C** Carious STE samples at h1–h3. **D** is a detailed view at ×1,000 original magnification of the area marked in **C**. **E**–**G** Noncarious STE samples at h1–h3. **F** Template superimposed for analysis. **H** is a detailed view at ×1,000 original magnification of the area marked in **G**. Adhesive surfaces are marked a, dentine surfaces are marked d, and composite surfaces are marked c.

After shear bond strength testing for the NonRF/deep caries lesion, the sample body was ground further until the pulp chamber was opened in the region of interest or close to it. At this level, a final height measurement (h4) was made, documenting the distance between the roof of the pulp chamber and the base of the sample and allowing the calculation of the height above the pulp for each of the levels h1–h3, retrospectively.

Fluorescence Image Analysis

Fluorescence images taken on surfaces h1–h3 prior to restoration placement were analysed using Fiji ImageJ [Schindelin et al., 2012]. A circular region of interest of 2-mm diameter was placed centrally over the lesion in the area at h1 where the restoration was to be placed. The mean red/green ratio per pixel for the region of interest was calculated and recorded as RG-mean for each sample. The region of interest was superimposed on subsequent images at h2 and h3 to allow repeated analysis of the same area at the three dentine depths for each sample.

Fracture Mode Analysis

The fracture surface of each composite cylinder (n = 345) was imaged in an SEM (FEI Quanta 400 FEG; Thermo Fisher Scientific, FEI Deutschland GmbH, Frankfurt am Main, Germany). One image of the fracture surface of each composite cylinder was taken at ×130 original magnification with analysis of material contrast low-vac mode, pressure 0.95 Torr, accelerating voltage 15 kV, spot size 3, solid-state backscatter detector, working distance ~10 mm, 30 µm aperture. In cases of uncertainly about the material visible on the fracture surface, additional images up to ×2,000 original magnification were made. A template allowing the fracture surface to be divided into 13 equally sized fields for analysis was placed on the ×130 SEM image; see Figure 3F. Each field was scored as one of the following 4 fracture modes: adhesive fracture (adhesive surface visible), cohesive fracture in dentine (dentine visible), cohesive fracture in composite (composite visible), or artifact (none of the above, mostly air bubbles). When more than one type of fracture surface was visible in the field, it was classified according to which type was the largest. In case of uncertainties, greater original magnification up to ×2,000 was used.

Statistical Analyses

All collected data were exported to SPSS (SPSS for Windows, version 29; SPSS Inc., Chicago, USA) for statistical processing. The data were descriptively presented as medians with 25–75% quantiles, minimum and maximum or as frequencies. Differences between groups for shear bond strength, height above the pulp, and fluorescence intensity for both carious and sound were statistically analysed using either the Mann-Whitney U test (independent variables) or the Wilcoxon signed-rank test (dependent variables). The level of significance was set at $p < 0.05$. The overall influence of variables was tested using the error rate method.

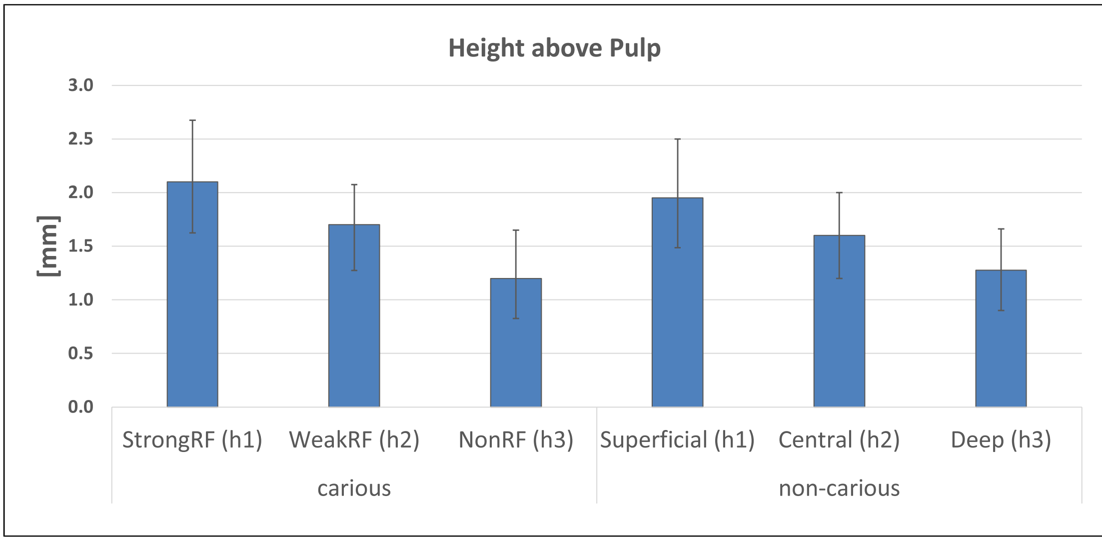

Fig. 4. Median and 25–75% quantiles (whiskers) for height remaining above the pulp roof in carious and noncarious samples at heights h1, h2, and h3. Differences for each height h1, h2, and h3 when comparing carious with noncarious samples were not statistically significant.

Results

Height over Pulp

Height remaining above the pulp for each fluorescence level and each height in noncarious dentine is given in Figure 4. Differences for each height h1, h2, and h3 when comparing carious with noncarious samples were not statistically significant.

Shear Bond Strength

An overview of the median shear bond strength for each of the adhesives tested at each depth in dentine is shown in Figure 5a for carious samples and Figure 5b for noncarious samples. Table 1 gives median, 25–75% quantiles (Q1, Q3), minimum and maximum for each adhesive at each depth in dentine for carious and noncarious samples. In carious dentine, the error rate method showed a significant influence for depth independent of the adhesives used. In noncarious dentine, this was not the case. The error rate method showed a significant influence for carious versus noncarious samples independent of depth or the adhesives used.

Carious Samples

On StrongRF, shear bond strength was highest for the self-etching adhesives SSE (median M = 14.4 MPa) and CSE (M = 10.2 MPa) followed by STE (M = 9.1 MPa) and lowest for OTE (M = 6.8 MPa). Only OTE and SSE were significantly different ($p \leq 0.05$). Shear bond strength increased significantly for all adhesives on WeakRF, compared to StrongRF. SSE (M = 19.8 MPa) was slightly higher than STE (M = 19.5 MPa) followed by CSE (M = 17.5 MPa) and OTE (M = 15.8 MPa). On WeakRF, there was no significant difference in shear bond strength between any of the adhesives. On NonRF, shear bond strength increased again for all adhesives. STE (M = 25.8 MPa) was highest followed by SSE (M = 22.4 MPa), CSE (M = 22.1 MPa), and OTE (M = 19.2 MPa). For STE and CSE, bond strength was significantly higher on NonRF compared to WeakRF.

Noncarious Controls

On noncarious dentine, shear bond strength values were very similar at all three dentine depths (Fig. 5b). Superficially and in deep dentine, the highest values (median) were achieved using OTE followed by STE and then CSE with SSE having the lowest shear bond strength for both superficial and deep dentine. In central dentine, the results were very similar except that STE rather than OTE achieved the highest values.

Regarding the differences between carious and noncarious dentine, shear bond strength was significantly lower on StrongRF than on superficial noncarious dentine for all adhesives except SSE. At the central depth, there were no significant differences between bond strengths achieved on WeakRF compared to central noncarious dentine. Bond strengths were significantly higher on NonRF compared to deep noncarious dentine

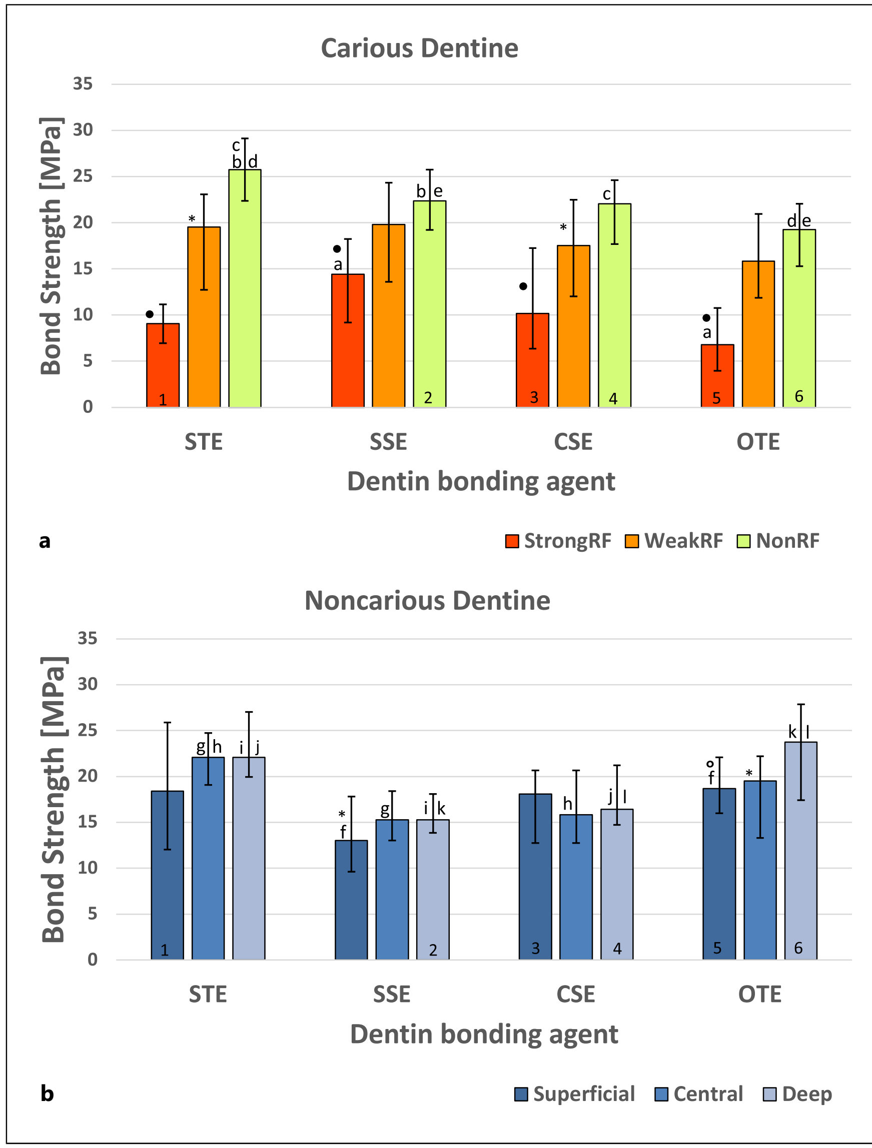

Fig. 5. Median (25–75% quantiles) shear bond strength for each adhesive in MPa for carious samples on StrongRF, WeakRF, and NonRF (**a**) and noncarious samples on superficial, central, and deep dentine (**b**). Columns marked with the numbers 1–6 indicate statistically significant differences when comparing carious with noncarious samples for the same adhesive at the same excavation depth (h1/h2/h3). Columns marked with the letters a–e indicate significant differences between adhesives at the same excavation depth in carious samples. Columns marked with the letters f–l indicate significant differences between adhesives at the same excavation depth in noncarious samples. Columns marked ● are significantly different to the next deepest and also to the deepest level. Columns marked * are significantly different to the next deepest level only. The column marked ° is significantly different to the deepest level.

for SSE and CSE. In contrast, OTE had significantly higher bond strength on deep noncarious dentine compared to NonRF.

Fracture Mode Analyses

Figure 3 shows exemplary SEM images of STE samples at h1–h3 on carious and noncarious dentine. These show the typical appearance of dentine, composite, and adhesive as seen on the fracture surfaces. The proportions of each fracture mode recorded for each adhesive on carious and noncarious samples are given in detail in Figure 6a and b. In general, adhesive failure made up the greatest proportion of fracture modes seen.

- STE: In noncarious samples, adhesive failure was the most common fracture mode, followed by cohesive failure in composite. In carious samples in StrongRF and WeakRF, the proportion of cohesive failures in dentine was greater, but in NonRF the cohesive failures in composite made up 50% of fracture mode, while cohesive failures in dentine remained the same.
- SSE: In noncarious samples, adhesive failure was by far the most common failure mode seen, but in carious samples cohesive failures in dentine and in composite were seen more frequently at all depths. This was most marked in StrongRF, where these made up 57.1% of the total.

Table 1. Bond strength in MPa for each adhesive at each depth in dentine for carious and noncarious samples

	Bond strength in MPa					
	adhesive	median	minimum	Q1	Q3	maximum
Carious						
StrongRF	STE	9.1	2.8	6.9	11.2	18.1
	SSE	14.4	5.7	9.2	18.2	22.1
	CSE	10.2	3.4	6.4	17.3	25.5
	OTE	6.8	1.1	4.0	10.8	18.1
WeakRF	STE	19.5	5.1	12.7	23.1	24.3
	SSE	19.8	9.1	13.6	24.3	30.0
	CSE	17.5	6.8	12.0	22.5	27.2
	OTE	15.8	4.5	11.9	20.9	28.3
NonRF	STE	25.7	19.8	22.4	29.1	33.4
	SSE	22.4	13.0	19.2	25.7	30.0
	CSE	22.1	10.8	17.7	24.6	34.0
	OTE	19.2	11.3	15.3	22.1	27.7
Noncarious						
Superficial	STE	18.4	5.7	12.0	25.9	34.5
	SSE	13.0	4.0	9.6	17.8	22.1
	CSE	18.1	7.4	12.7	20.7	28.9
	OTE	18.7	12.4	16.0	22.1	26.6
Central	STE	22.1	12.4	19.1	24.8	28.3
	SSE	15.3	11.9	13.0	18.4	20.4
	CSE	15.8	9.1	12.7	20.7	28.3
	OTE	19.5	7.9	13.3	22.2	24.9
Deep	STE	22.1	13.0	19.9	27.0	30.0
	SSE	15.3	1.7	13.9	18.1	24.9
	CSE	16.4	10.8	14.7	21.2	27.7
	OTE	23.8	14.7	17.4	27.9	31.7

- CSE: A small increase in the rates at all depths for cohesive failures in both composite and dentine was seen when comparing carious with noncarious samples, but adhesive failure was still the most common failure mode.
- OTE: Adhesive failure was the most common mode recorded for OTE, with very similar distribution of fracture modes at all 3 depths in both carious and noncarious samples.

Fluorescence Analyses

An overview of the mean red/green pixel intensity ratio for samples at each of the depths in dentine is given in Figure 7. The values for the carious samples range from just below 1 to 3, while those for the noncarious samples remain well below 1. The red-to-green fluorescence ratio was highest in the strongly red fluorescing layer compared to central weak red-fluorescing dentine and lowest in deep non-fluorescing dentine. In noncarious samples, the red/green ratio remains consistent across all depths in dentine. Differences in fluorescence intensity at each height when comparing carious with noncarious samples were statistically significant.

Discussion

This study compared the effect of selective removal of caries to different depths in dentine, determined using visible autofluorescence, on shear bond strength and fracture mode of 4 different adhesive approaches. Samples with natural caries rather than artificially created lesions were chosen because bacterially produced porphyrin is necessary for the specific red autofluorescence found in carious dentine [Buchalla et al., 2008; Lennon et al., 2022]. We excluded samples with very deep lesions likely to have resulted in pulp exposure. The samples were evenly distributed among the groups according to both lesion size, determined radiographically, and also lesion location. Noncarious-matched controls were used with excavation to similar depths in dentine as in the carious group to observe differences in adhesive performance in sound dentine compared to affected tissue.

Grinding flat was chosen as a method for caries removal rather than cavity preparation in this case to allow testing restorations at 3 different depths in the same tooth while also producing a flat surface suitable for shear

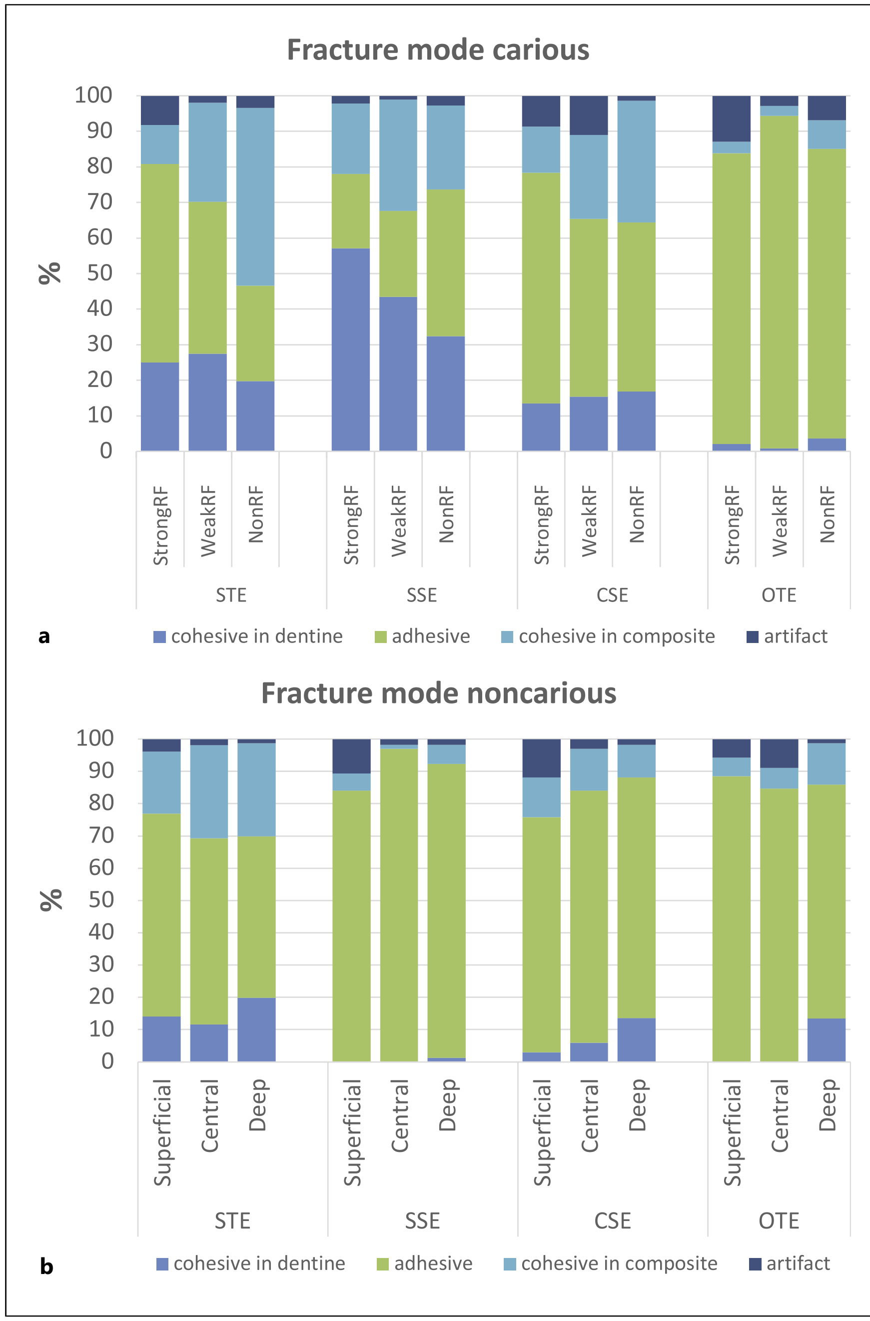

Fig. 6. Proportion of each of the fracture modes (cohesive in dentine, adhesive, cohesive in composite, and artifact) at each of the 3 depths in dentine in carious (**a**) and noncarious (**b**) samples.

bond testing with a dentine smear layer as found following conventional preparation [Buchalla et al., 2007]. The adhesive approaches were chosen on the basis that they are the gold standards available at present for dentine bonding and included universal, self-etch, and total etch approaches.

Both Scotchbond Universal and CSE contain 10-methacryloyloxydecyl dihydrogen phosphate (MDP) monomer which is known to have chemical bonding capacity to metal alloys and zirconia, but also to calcium [Fukegawa et al., 2006; Nagaoka et al., 2017], which most other monomeric components from dentine adhesives, such as HEMA or TEGDMA, have not. Since carious dentine contains more calcium in deeper less demineralized, and less red-fluorescing layers, but less calcium in superficial, more red-fluorescing layers, the said calcium-binding property of MDP may increase dentine bond, particularly in layers expected to have higher calcium content.

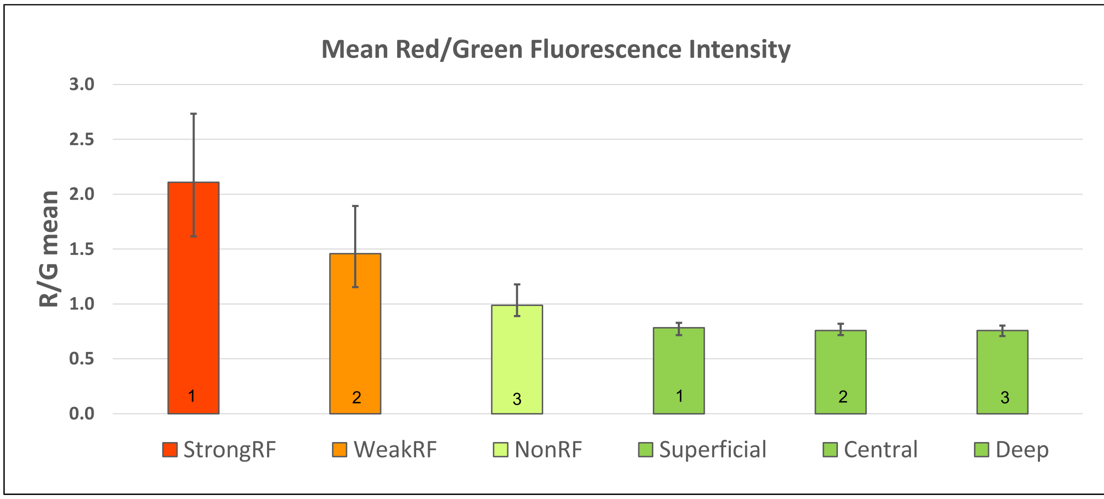

Fig. 7. Mean ratio of red/green fluorescence intensity for carious samples with StrongRF, WeakRF, and NonRF and noncarious samples at superficial, central, and deep depths in dentine. Columns marked with the numbers 1–3 indicate statistically significant differences when comparing carious with noncarious samples at the same excavation depth (h1/h2/h3).

OptiBond FL, as a representative of etch-and-rinse adhesive systems, requires phosphoric acid etching of dentine to achieve a strong dentine bond [De Munck et al., 2003]. However, the phosphoric-etching step may be too "aggressive" for previously demineralized dentine present in more superficial layers of carious dentine, leading to reduced bond strength values [Nakajima et al., 2011]. If classical self-etch adhesives, such as CSE, are used, it is known that prior acid etching reduces the bond strength to sound dentine [Van Landuyt et al., 2006]. This problem has been overcome with the introduction of universal adhesives, which typically comprise MDP or similar molecules, but have shown good dentine bonding capability not only in self-etch mode but also when used in etch-and-rinse mode [Yamauchi et al., 2019]. For this reason, we included these three adhesive systems and applied Scotchbond Universal in both etch-and-rinse and self-etch modes.

Our results confirm this, with the universal adhesive in self-etch mode (SSE) producing significantly higher bond strengths compared to OTE on StrongRF. On noncarious dentine, the opposite is true; OTE produces significantly higher bond strength compared to the universal adhesive used in self-etch mode (SSE). These differences are most likely due to the lower mineral content of affected dentine compared to sound and differences in the resultant hybrid layer formed. In cases where StrongRF dentine can or should not be removed to avoid pulp exposure, a universal adhesive in self-etch mode may be the best choice.

All adhesives tested had significantly higher bond strength on WeakRF compared to StrongRF, indicating that it is advisable to remove StrongRF for this reason. However, further excavation to NonRF only improved bond strength significantly for STE and CSE meaning that for SSE and OTE no improvement in bond strength is gained by removing red-fluorescing dentine completely. STE performed equally well on affected compared to noncarious dentine at this depth.

Tensile bond strength to carious dentine has been shown to be lower than on sound dentine for a variety of adhesives [Nakajima et al., 1995]. Scholtanus et al. [2010] found no significant difference in microtensile bond strength for CSE used in normal compared to caries-affected dentine, whereas other groups have shown no significant difference in bond strength between self-etch and total etch modes for universal adhesives used on caries-affected dentine [Hass et al., 2019].

Interestingly, in the present study, bond strength was significantly higher for adhesives used in self-etch mode (SSE and CSE) on the deepest layer of affected dentine (NonRF) compared to a similar height in noncarious dentine, highlighting that the caries process affects even deeper layers with respect to dentine bonding. Our findings show no difference in bond strength for Scotchbond Universal according to etching mode, when used in superficial sound dentine, which agrees with previous research [Yamauchi et al., 2019]. But in deeper layers of sound dentine, the total etch version had significantly better values, similar to results by Hass et al.

[2019]. These differing results may be explained by dentine tubule anatomy which changes markedly approaching the pulp.

Further information on the performance of each adhesive was obtained from the fracture mode analysis. SEM in low-vac mode allows the native material contrast to be viewed to differentiate between materials on the sample surfaces [Scholz et al., 2021]. Cohesive failure in dentine or composite was easily identified at ×130 original magnification. The appearance of adhesive was quite varied, depending on which adhesive was used and whether the fracture was at the interface to composite or dentine or in adhesive or various parts of the hybrid layer. Surfaces were therefore viewed in higher magnifications up to ×2,000 for clarification. Therefore, classification as "mixed" fracture, which is often used, could be avoided [Kensche et al., 2016]. In noncarious samples, cohesive failures in dentine were not found for STE or OTE in the superficial or central layers. Very few were found in deep noncarious dentine. In carious tissue, however, SSE produced a large proportion of cohesive failures in dentine. Cohesive failures in dentine were also increased for STE and CSE. This, although the bond strengths were quite good, may indicate differences in hybrid layer formation between the two types of dentine [Nakajima et al., 2011]. The main reason may be the lower cohesive properties of the affected dentine itself as a result of the higher degree of demineralization. Cohesive failures in composite were also seen more frequently for MDP-containing adhesives (STE, SSE, and CSE) in carious dentine compared to noncarious.

Caries was removed down to all but a hint of yellow/orange fluorescence, so that we are at the bottom of the fluorescent part of the lesion and not beyond it. At this point, the most infected or contaminated tissue has been removed but dentine is often demineralized compared to sound tissue. Also, at the advancing front of the lesion the degree of red fluorescence gives way to an orange or yellow appearance while the green fluorescence here is much brighter than in normal dentine. The fluorophore, which causes green autofluorescence in dentine, has not yet been identified but is thought to be organic rather than inorganic [Kvaal and Solheim, 1989]. Increased intensity of green autofluorescence within less mineralized areas of healthy teeth and in artificially demineralized dentine has already been described [Foreman, 1980; van der Veen and ten Bosch, 1996]. Van der Veen and ten Bosch [1996] suggested that fluorescence is quenched when the fluorophore is linked to hydroxyapatite and that when the tissue is demineralized, de-quenching occurs, making that area appear brighter.

Individual sensitivity to red may be subjective, but, in general, if the ratio of red to green intensity is greater than 1 an object will appear red, or if it is below 1 that object will be seen as green. Objective R/G values recorded from the carious and noncarious samples in this study agree with the visual impression of the samples as strong red fluorescence, weak red fluorescence, and non-red fluorescence in the carious samples, while the sound dentine samples all have an R/G value well below 1 and are perceived as green.

This study is limited in that it was carried out in vitro, on flat specimens, and clinical conditions could not be completely simulated. Information about the longevity of restorations placed following selective removal of fluorescent caries should be investigated clinically in the future. Only one material from each material class was tested. More adhesives with different compositions are commercially available, and it is possible that different materials might produce different results.

Conclusions

Within the limitations of this study, it can be concluded that removal of StrongRF dentine results in a surface that allows both etch-and-rinse (STE, OTE) and self-etching (SSE and CSE) adhesives to produce a better bond strength, than if it is not removed. When it is advisable to leave strongly fluorescent dentine on the cavity floor, for example, to avoid risk of pulp exposure, a universal adhesive used in self-etch mode (SSE) may provide the best bond strength. But it must be remembered that long-term clinical success of adhesive restoration depends not only on bond strength but also on a variety of other factors.

Statement of Ethics

The use of extracted teeth from anonymous human donors was approved by the University of Regensburg Ethics Committee (Reference: 19-1327-101). Verbal informed consent was obtained from all donors of extracted teeth prior to the study. This consent procedure was reviewed and approved by the University of Regensburg Ethics Committee approval number 19-1327-101, date of decision February 20, 2019.

Conflict of Interest Statement

Á.M.L. and W.B. were appointed inventors on a now-expired US patent for a fluorescence-aided caries excavation technique held by Indiana University.

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Author Contributions

Conception and design: Wolfgang Buchalla and Áine Lennon. Data acquisition: Nina Sophie Reich, Áine Lennon, Gerlinde Ferstl, and Helga Ebensberger. Data analysis and interpretation: Nina Sophie Reich, Áine Lennon, K.-A. Hiller, and Wolfgang Buchalla. Drafted the manuscript: Áine Lennon and Nina Sophie Reich. Revised the manuscript: Áine Lennon, Wolfgang Buchalla, Nina Sophie Reich, K.-A. Hiller, Gerlinde Ferstl, and Helga Ebensberger.

Data Availability Statement

All data generated or analysed during this study are included in this article. Further enquiries can be directed to the corresponding author.

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