Influence of Fluoride Varnish Application on Enamel Adhesion of a Universal Adhesive

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Purpose: To study the effect of the varnish type, application time and surface polishing on the shear bond strength (SBS) of a universal adhesive in healthy and demineralized bovine enamel.

Materials and Methods: 432 bovine primary central incisors were assigned to 18 groups according to enamel mineralization [healthy and demineralized], topical varnish [Clinpro White Varnish (CWV; 3M Oral Care) and Profluorid (PFV, Voco)], remineralization time [24 h or 21 days] and polishing or not of the enamel surface. Adhesion was tested using Futurabond M (Voco)+ and GrandioSO (Voco). Sheer bond strength (SBS) was measured and the fracture mode studied. The statistical analysis was performed using two-way ANOVA, Tukey's test, and Pearson's chi-squared test.

Results: In healthy bovine enamel, CWV reduced SBS at 24 h and 21 days; polishing significantly improved SBS. PFV increased SBS in healthy enamel at 21 days and demineralized enamel at 24 h and 21 days; polishing had no effect on SBS. The application time and polishing of the enamel surface affected the behavior of varnishes with respect to SBS. There was a correlation between the type of fracture and the degree of mineralization as well as the timepoint of varnish application.

Conclusions: Remineralization of demineralized enamel with fluoride varnishes permits the recovery of the bond strength obtained in healthy enamel. Of the two varnishes studied, PFV had the highest SBS and more uniform behavior, regardless of the application timepoint, degree of mineralization, and surface treatment of the enamel.

Keywords: demineralization, enamel, remineralization, fluoride varnish, tricalcium phosphate, shear bond strength.

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In 2015, untreated dental caries in permanent teeth was the most prevalent disease worldwide, affecting 2.5 billion people, with the primary dentition of 573 million children also affected.[21] Caries is a widespread chronic childhood disease, and the prevalence has increased in recent years, reaching 23% in US children ages 2–5 years.[27]

The caries process is initiated by a shift in the demineralization/remineralization balance in favor of demineralization, due to acids produced by bacterial metabolism of dietary carbohydrates in the plaque biofilm, resulting in a net loss of calcium and phosphate ions from the underlying enamel surface.[15] These incipient noncavitated lesions can develop into cavitated lesions over time if this process is left unchallenged.

Interventions in the caries process are of two types: (a) disruption of the caries process, or patient-level intervention

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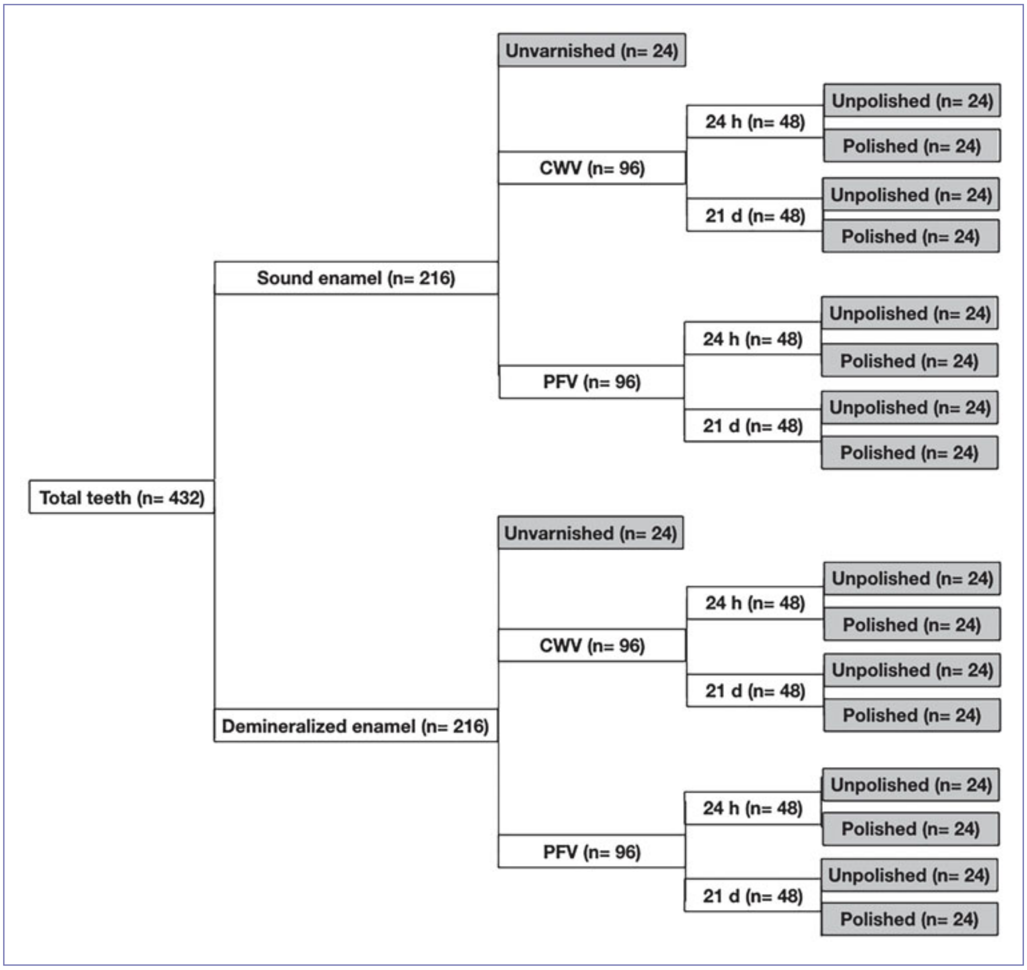

Fig 1 Experimental groups.

(preventive dietary advice, oral hygiene/biofilm control advice)[3,28] and (b) lesion-level interventions that include non-/micro-invasive and/or minimally-invasive treatments.[16,28] Noninvasive therapies can arrest the caries process and repair the initial enamel lesions, thus minimizing the loss of tooth structure.[34,28] To arrest or reverse early noncavitated carious lesions on the proximal, facial, or lingual surfaces of primary and permanent teeth, the American Dental Association suggests using 5% NaF varnish.[30]

Dental topical varnishes have been shown to release calcium and fluoride ions to some degree, but not all release phosphate ions. During remineralization, the crystalline phase formed depends on the availability of fluoride, calcium and phosphate ions. Fluoride, which is essential for remineralization, is stored as calcium fluoride pairs (CaF^+). High inorganic phosphate levels promote the formation of poorly soluble fluoride phases that may decrease bioavailability of the fluoride ion.[29] Clinpro White Varnish (CWV, 3M Oral Care; St Paul, MN, USA) contains functionalized calcium phosphate and fluoride, and releases large amounts of inorganic phosphate ions that reduce the bioavailability of fluoride ions necessary for remineralization.[13] Profluorid (PFV, Voco; Cuxhaven, Germany) contains 5% sodium fluoride suspended in a mostly water-free varnish base, and has shown ranges of fluoride release and deposition on enamel comparable to that of CWV.[7]

The latest generations of dental adhesives used in restorative dentistry have been evaluated with respect to the effect of fluoride on their bond strength. Some studies suggest that the same mechanism by which fluoride decreases demineralization increases the enamel resistance to acid etching and thus decreases the interfacial bond strength.[1] Others suggest that fluoride does not affect the bond strength of the adhesive to enamel with etch-and-rinse adhesives,[14] while yet others have observed an increase.[22] In all these studies, bond strength was measured using shear bond testing.[24]

In childhood and in patients of all ages with a medium to high risk of caries, initial carious lesions can progress rapidly to cavitation.[16] This scenario can be prevented using 5% fluoride varnishes at each maintenance or recall visit, to help repair the initial enamel surface damage and minimize the loss of tooth structure.[28] When progressed cavitated lesions are prepared for restoration with resin composite, according to minimally invasive operative principles, tissue preservation is maximized which requires adhesive bonding to potentially remineralized enamel. Of the few studies that have evaluated bonding to remineralized enamel with sodium fluoride varnishes, most have been carried out with orthodontic brackets on sound tissues.

Therefore, the primary objective of this study was to evaluate the effect of two 5% fluoride varnishes (containing 22,600 ppm fluoride), with and without functionalized tricalcium phosphate, on the adhesion of a universal adhesive to enamel.

The null hypothesis was that the shear bond strength (SBS) of a universal adhesive on bovine enamel treated with a fluoride varnish would not be affected by the enamel surface condition (healthy vs demineralized enamel), varnish type (sodium fluoride or sodium fluoride plus modified calcium phosphate), timepoint of varnish application (24 h vs 21 days), or the surface treatment of the enamel (polished vs unpolished).

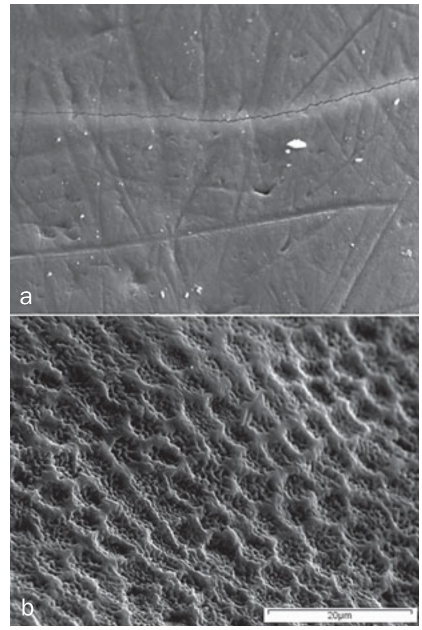

Fig 2 Representative SEM images of enamel (2000X). a. Healthy enamel. b. Demineralized enamel with a very porous appearance, typical of acid attack.

MATERIALS AND METHODS

Teeth

Four hundred thirty-two primary central mandibular bovine incisors age < 1 year, whose vestibular surfaces showed no visible fractures or defects caused by extraction, were obtained from an industrial slaughterhouse (El Cabezo; Murcia, Spain). All incisors selected presented a flattened labial surface sufficient for the correct positioning of the polyethylene cylinder where the resin composite was to be placed. The study was approved by the ethics committee on animal experimentation of the University of Murcia (code: 261/2015).

After extraction, teeth were cleaned of organic debris, washed and immersed in a 0.1% thymol solution for 24 h. Subsequently, they were stored in distilled water, refreshed daily. The teeth were stored no longer than six months post-extraction according to ISO standards (TS 11405:2015).[19] Before use, the vestibular surfaces of all teeth were checked for any defects at 40X magnification (SMZ-U Stereoscopic Zoom Microscope, Nikon, Tokyo, Japan). All teeth without defects were cleaned with a rubber cup (Rubber polishing cups, Produits Dentaires; Vevey, Switzerland) and mounted on a counter-angle rotary handpiece (5000 rpm, INTRAcompact 2068CHC, KaVo; Biberach, Germany) under water.

Experimental Groups

The 432 incisors were assigned to 18 experimental groups of 24 incisors each, using a randomization table. Twenty incisors were reserved for the SBS test and four for observation of the surface by scanning electron microscopy (SEM). The experimental groups were: (1) healthy enamel; (2) healthy enamel with CWV (3M Oral Care) at 24 h; (3) healthy enamel with CWV and polished at 24 h (4) healthy enamel with PFV (Voco; Cuxhaven, Germany) at 24 h; (5) healthy enamel with PFV and polished at 24 h; (6) healthy enamel with CWV at 21 days; (7) healthy enamel with CWV and polished at 21 days; (8) healthy enamel with PFV at 21 days; (9) healthy enamel with PFV and polished at 21 days; (10) demineralized enamel; (11) demineralized enamel with CWV at 24 h; (12) demineralized enamel with CWV and polished at 24 h; (13) demineralized enamel with PFV at 24 h; (14) demineralized enamel with PFV and polished at 24 h; (15) demineralized enamel with CWV at 21 days; (16) demineralized enamel with CWV and polished at 21 days; (17) demineralized enamel with PFV at 21 days; (18) demineralized enamel with PFV and polished at 21 days (Fig 1).

Demineralization/Remineralization of Enamel

Static demineralization was carried out by immersing teeth in a demineralizing solution composed of 2.2 mM calcium chloride ($CaCl_2 \cdot 2H_2O$), 2.2 mM monosodium phosphate ($NaH_2PO_4 \cdot 7H_2O$), and 0.05 M lactic acid for 48 h.[25] pH was adjusted to 4.5 with 50% sodium hydroxide (NaOH). Enamel demineralization was verified using SEM (Fig 2).

In groups with healthy enamel and those with demineralized enamel, remineralization was performed by covering the entire enamel surface with a uniform layer of CWV or PFV varnish using a brush applicator, as per manufacturer's instructions. The composition of the varnishes is shown in Table 1, together with the other materials used in the study.

The 24-h groups received varnish on day 0. The 21-day groups received varnish on days 0, 7, 14, and 21. To keep teeth hydrated, they remained submerged in artificial saliva throughout the experimental procedure at a temperature of 37°C in an incubator (Digitheat-TFT, JP Selecta; Barcelona,

Table 1 Product composition according to material safety data sheets (MSDS)

Product	Composition	%	Instructions for use
Dentaflux	Orthophosphoric acid	37	Etch for 20 s. Wash with an abundant water spray for 20 s. Dry the surface.
	Excipients	63	
Futurabond M+	2-hydroxyethyl methacrylate (HEMA)	10–25	Rub the surface for 20 s. Dry the adhesive layer with dry, oil-free air for 10 s. Light cure adhesive layer for 10 s using a polymerization lamp.
	Bis-GMA	10–25	
	Ethanol	10–25	
	Adhesive monomer acid	2.5–5	
	Urethane dimethacrylate	2.5–5	
	Catalyzer	< 2.5	
	Pyrogenic silicic acids	< 2.5	
GrandioSO	Bis-GMA	2.5–5	Apply in 2-mm-thick layers. Polymerize each layer for 20 s.
	Triethylene glycol dimethacrylate	2.5–5	
	Bis-EMA	2.5–5	
	EBPADA	–	
	DMABE	–	
Clinpro White Varnish	Pentaerythritol glycerol ester of colophony resin	30–75	Eliminate any excess saliva from the vestibular tooth surface. Apply the varnish as a thin film covering the entire surface. Place the tooth in saliva to ensure proper setting of varnish.
	N–hexane	10–15	
	Ethyl alcohol	1–15	
	Sodium fluoride	1–5	
	Flavor enhancer	1–5	
	Thickener	1–5	
	Food grade flavoring	1–5	
	Modified tricalcium phosphate	<5	
Profluorid Varnish	Ethanol	10–25	Eliminate any excess saliva from the vestibular tooth surface. Apply the varnish as a thin film covering the entire surface. Place the tooth in saliva to ensure proper setting of varnish.
	Sodium fluoride	2.5–5	

Spain). The artificial saliva was renewed daily until adhesion, which was performed on days 1 and 22. The artificial saliva was composed of 1% carmellose sodium, 13% sorbitol, 0.12% potassium chloride, 0.084% sodium chloride, 0.005% magnesium chloride hexahydrate, 0.015% anhydrous calcium chloride, 0.017% dibasic potassium phosphate, and 0.1% Nipagin sodium. The pH was adjusted to 6.57.[36]

Scanning Electron Microscopy (SEM)

The enamel surfaces of four teeth per group were examined by SEM. The incisor roots were removed using a diamond disk (Komet Dental, Gebr. Brasseler; Lemgo, Germany) mounted on a handpiece and with abundant water cooling. The crowns were cleaned with a spray of distilled water, dried, and left at room temperature for 24 h for dehydration. They were then placed on plates, the surfaces to be observed were gold sputter-coated (E-5100, Bio-Rad Laboratories; Hercules, CA, USA) and placed in a vacuum hood at a voltage of 2.5 kV and an intensity of 20 mA. The coating time to which each plate was subjected was 4 min. The teeth were examined using SEM (JSM-6100, JEOL; Tokyo, Japan) and observed at 20 kV. Image acquisition was made using the INCA microanalysis suite (Oxford Instruments; Abingdon, Oxfordshire, England) digital imaging program. Representative images were selected for further study.

Adhesive Procedure

The vestibular surfaces of all teeth were dried with dry compressed air. In the groups where the varnished enamel was polished, this was done lightly using a nylon brush (Medicaline; Castellón, Spain), without polishing paste, mounted on a slow-speed rotary handpiece, in order to not affect the enamel surface. The polished teeth were immersed in an ultrasonic bath for 30 min to remove the varnish residue from the enamel surface. The area to be bonded was etched for 20 s with orthophosphoric acid 37% (DentaFlux acid, DentaFlux; Madrid, Spain), washed with an abundant spray of water for 20 s, and dried until

Table 2 Mean shear bond strength (MPa) and standard deviation (SD) of healthy enamel groups: CWV (Clinpro White Varnish) and PFV (Profluorid Varnish)

CWV		PFV	
Group	SBS ± SD	Group	SBS ± SD
Unvarnished	40.9 ± 6.3	Unvarnished	40.9 ± 6.3
24 h	31.1 ± 10.7[a]	24 h	41.9 ± 8.2
24 h, polished	39.7 ± 7.9[#]	24 h polished	39.8 ± 9.2
21 days	24.1 ± 8.7[a]	21 days	46.± 4.2[a,b]
21 days, polished	32.5 ± 8.8[a,#]	21 days, polished	46.7 ± 6.8[a,b]
[a]p < 0.05 vs unvarnished; [b]p < 0.05 vs 24h polished; [#]p < 0.05 vs unpolished.			

Table 3 Mean shear bond strength (MPa) and standard deviation (SD) of demineralized enamel groups: CWV (Clinpro White Varnish) and PFV (Profluorid Varnish)

CWV		PFV	
Group	SBS ± SD	Group	SBS ± SD
Unvarnished	33.4 ± 7.8	Unvarnished	33.4 ± 7.8
24 h	25.1 ± 10.3[a]	24 h	44.1 ± 13.7[a]
24 h, polished	38.2 ± 5.0[#]	24 h, polished	41.6 ± 11.9[a]
21 days	23.7 ± 8.3[a,b]	21 days	43.4 ± 7.1[a]
21 days, polished	30.9 ± 9.9[b,#]	21 days, polished	41.2 ± 7.3[a]
[a]p < 0.05 vs unvarnished; [b]p < 0.05 vs 24h polished; [#]p < 0.05 vs unpolished.			

the surface was a matte white color. Futurabond M+ adhesive (Voco) was placed with a disposable applicator, rubbing the surface for 20 s. The solvent was removed with an oil-free, dry blast of air for 10 s and polymerized (SmartLite LED lamp, Dentsply Sirona; Konstanz, Germany) at 1250 W/cm^2 for 10 s. A3 color GrandioSO resin composite (Voco) was placed in two 2-mm-thick layers using a polyethylene cylinder with an internal diameter of 3 mm (area 7.1 mm^2) and a height of 4 mm. Each layer was polymerized for 20 s. The samples were then stored at 37°C in distilled water for 24 h.[19]

Shear Bond Strength Testing

After 24 h, the incisors were mounted on cylinders with an internal diameter of 3 cm and a height of 4 cm, submerging the roots in type IV plaster. The SBS test was performed using a universal testing machine (AGS-1 KND, Shimadzu; Kyoto, Japan) exerting an inciso-cervical force perpendicular to the resin-composite/tooth-surface interface with a steel stem terminating in a 30-degree bevel. The load cell was 1 kN and the machine head speed was 1 mm/min.[19] The force required to produce debonding of the material was recorded in Newtons (N) and subsequently transformed into MPa by dividing it by the bonding area (7.1 mm^2) according to the formula MPa = N/mm^2.

Types of Fracture

The fracture surfaces were examined with a stereomicroscope (SMZ-U Stereoscopic Zoom Microscope, Nikon; Tokyo, Japan). The fracture mode was classified into one of four categories:[9] cohesive enamel fracture; cohesive resin composite fracture; adhesive fracture; mixed fracture (part cohesive and part adhesive failure).

Statistical Analysis

The statistical analysis was performed using the SigmaStat 3.5 statistical software package (Systat Software; Point Richmond, CA, USA). SBS were normally distributed (Kolmogorov-Smirnov test, $p>0.05$) and showed homogeneity of variance (Levene's test, $p>0.05$).

Intergroup comparisons were made using one-way ANOVA. When there were between-group differences, two-way comparisons were made using Tukey's test. Statistical significance was set at $p < 0.05$.

Two-way ANOVA (factors two by two) was used to analyze interactions between the study factors: enamel surface condi-

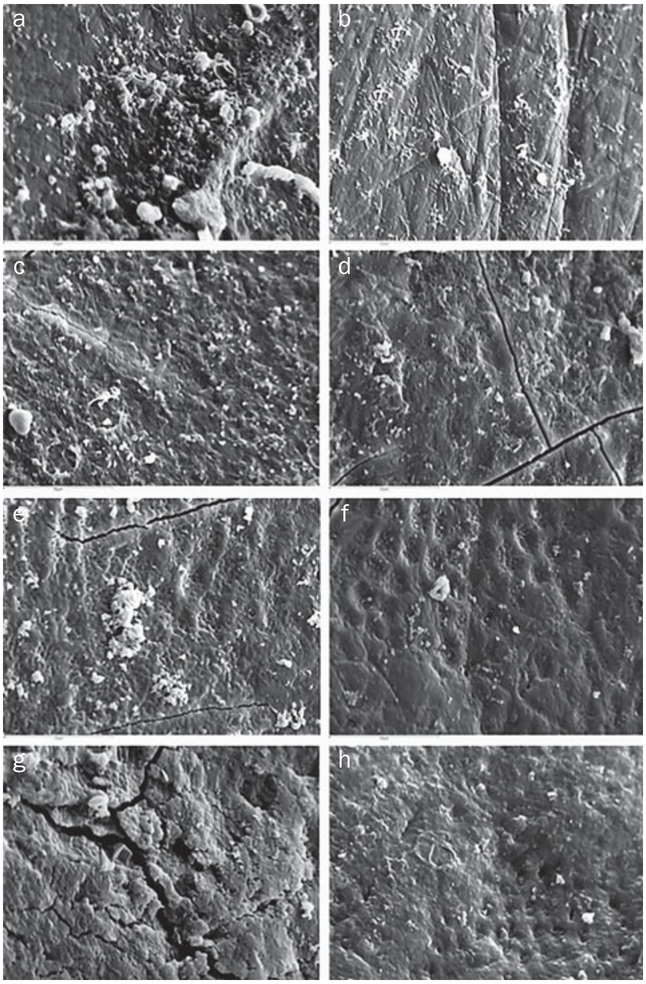

Fig 3 Representative SEM images of enamel surfaces treated with CWV (2000X). a. Varnished healthy enamel at 24 h. b. Varnished and polished healthy enamel at 24 h. c. Varnished healthy enamel at 21 days. d. Varnished and polished healthy enamel at 21 days. e. Varnished demineralized enamel at 24 h. f. Varnished and polished demineralized enamel at 24 h. g. Varnished demineralized enamel at 21 days. h. Varnished and polished demineralized enamel at 21 days. Unpolished samples show a homogeneous layer of varnish with agglomerates on the surface. Polished samples have exposed enamel surfaces. In demineralized groups, the porosities are covered, more at 21 days than at 24h.

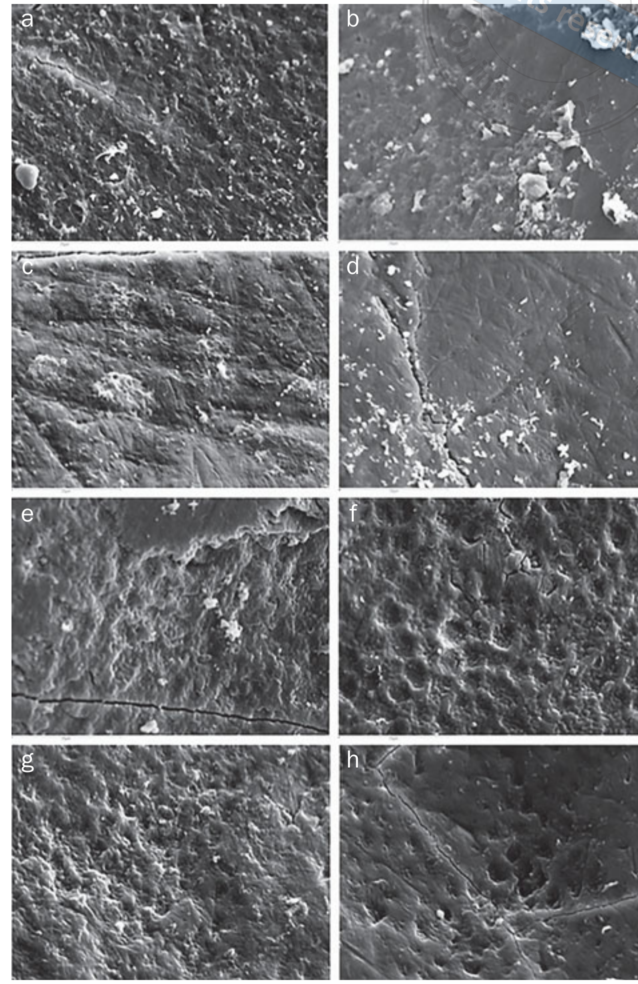

Fig 4 Representative SEM images of enamel surfaces treated with PFV (2000X). a. Varnished healthy enamel at 24 h. b. Varnished and polished healthy enamel at 24 h. c. Varnished healthy enamel at 21 days. d. Varnished and polished healthy enamel at 21 days. e. Varnished demineralized enamel at 24 h. f. Varnished and polished demineralized enamel at 24 h. g. Varnished demineralized enamel at 21 days. h. Varnished and polished demineralized enamel at 21 days. Unpolished samples show a homogeneous layer of varnish with agglomerates on the surface. Polished samples expose the enamel surface. In demineralized groups the porosities are covered, more at 21 days than at 24h.

tion (healthy vs demineralized enamel), varnish type (CWV vs PFV), time (24 h vs 21 days) and surface treatment (polished vs unpolished). To eliminate the possible effects of the different values of the control groups (healthy enamel without varnish and demineralized enamel without varnish), increments in the SBS compared with those of controls were evaluated.

To determine possible associations between the fracture modes and the experimental factors, contingency tables and Pearson's chi-squared test were used. Four contingency tables were constructed to analyze the following cross comparisons: type of fracture and enamel surface condition, type of fracture and type of varnish, type of fracture and time, and type of fracture and surface treatment. An α-error of 0.05 and a test power of 0.80 were accepted.

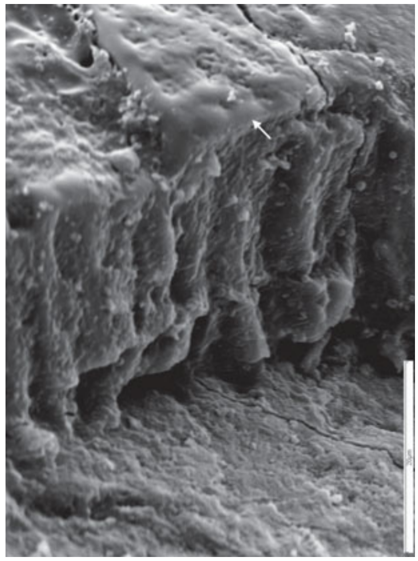

Fig 5 SEM image of CWV on demineralized enamel at 24 h. Arrow shows the varnish layer (2000X).

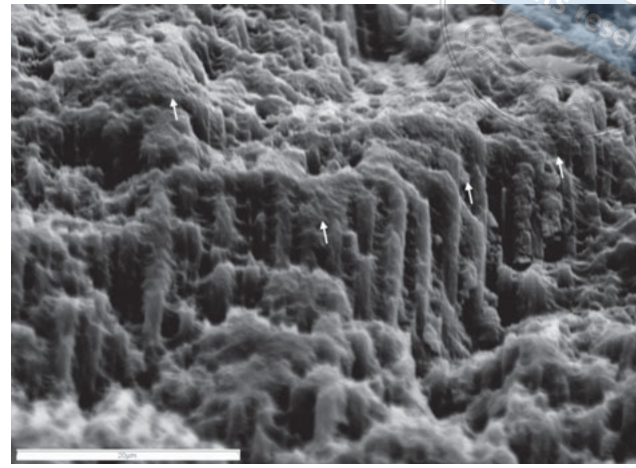

Fig 6 SEM image of enamel section showing the close apposition of varnish on the enamel surface and penetration into the interprismatic spaces (white arrows) (2000X).

RESULTS

Intergroup Comparisons

The mean SBS in unvarnished healthy enamel was 40.9 ± 6.3 (SD) MPa (Table 2). The SBS was influenced by the type of varnish. CWV produced a significant reduction at 24 h (31.1 ± 10.7 MPa; $p < 0.05$) and at 21 days (24.1 ± 8.7 MPa; $p < 0.05$). When the varnish layer was removed by light surface polishing, a significant recovery in SBS was observed, which reached the control value at 24 h (39.7 ± 7.9 MPa). At 21 days there was a significant increase in the unpolished SBS (32.5 ± 8.8 MPa; $p < 0.05$), but this did not reach the control value. PFV maintained the control SBS at 24 h in the unpolished (41.9 ± 8.2 MPa) and polished groups (39.8 ± 9.2 MPa) and a significant increase was observed at 21 days in the unpolished (46.8 ± 4.2 MPa; $p < 0.05$) and polished groups (46.7 ± 6.78 MPa; $p < 0.05$).

The unvarnished demineralized enamel group had an SBS of 33.4 ± 7.8 MPa (Table 3), significantly lower than healthy enamel ($p < 0.001$). Again, the effect of the varnishes on SBS differed. CWV produced a significant reduction in SBS at 24 h (25.1 ± 10.3 MPa; $p < 0.05$) and 21 days (23.7 ± 1.9 MPa; $p < 0.05$). When the varnish was removed, the values were recovered at 24 h (38.2 ± 5.0 MPa; $p < 0.05$) and at 21 days (30.9 ± 9.9 MPa; $p < 0.05$). PFV produced a significant increase in SBS at both 24 h and 21 days, regardless of whether the surface was polished or not.

Factor Analysis: Mineralization, Varnish Type, Time, Polishing

There was a significant interaction ($p < 0.001$) between the type of varnish used and the timepoint of application. The interaction was negative with CWV, decreasing the SBS at 24 h and 21 days (-3.6 MPa; -9.0 MPa, respectively). However, the interaction was positive with PFV, increasing the SBS at 24 h and 21 days (4.7 MPa; 7.4 MPa, respectively). There was a significant difference between the two varnishes at 24 h and 21 days.

There was a significant interaction between the type of varnish and surface treatment ($p < 0.001$). The interaction was positive with CWV in the sense that, in polished samples, the SBS reduction was -1.5 MPa compared with -11.1 MPa in unpolished samples. However, PFV affected the SBS equally regardless of whether the enamel surface was polished (5.2 MPa) or not (6.9 MPa).

Fracture Modes (Table 4)

There was an association between the type of fracture and the enamel surface condition ($X^2 = 11.11$; $p = 0.011$; power = 0.81). Healthy enamel most often exhibited adhesive fractures (55%), while cohesive enamel fractures (50%) were associated with demineralized enamel. When the demineralized enamel was remineralized, the proportion of cohesive enamel fractures fell (20%) and the proportion of adhesive fractures rose (70%).

An association also existed between the type of fracture and the timepoint at which the varnishes acted on the enamel ($X^2 = 21.65$; $p = 0.001$; power = 0.96). When varnish was applied at 24 h, there was a higher frequency of cohesive enamel fractures and fewer adhesive fractures. When varnish was applied at 21 days, there was a decrease in cohesive enamel fractures and an increase in adhesive fractures.

The type of fracture and the type of varnish were associated ($X^2 = 13.084$; $p = 0.042$; power = 0.780), and an association was observed between cohesive enamel fractures and PFV, and between adhesive fractures and CWV. For

Table 4 Fracture modes

Group	Cohesive failure in enamel	Cohesive failure in composite	Adhesive failure	Mixed failure
Healthy enamel unvarnished	20% (4/20)	15% (3/20)	60% (12/20)	5% (1/20)
Healthy enamel with CWV 24 h	40% (8/20)	–	40% (8/20)	20% (4/20)
Healthy enamel with CWV 24 h polished	50% (10/20)	15% (3/20)	30% (6/20)	5% (1/20)
Healthy enamel with CWV 21 days	5% (1/20)	10% (2/20)	75% (15/20)	10% (2/20)
Healthy enamel with CWV 21 days polished	25% (5/20)	–	75% (15/20)	–
Healthy enamel with PFV 24 h	20% (4/20)	–	75% (15/20)	5% (1/20)
Healthy enamel with PFV 24 h polished	25% (5/20)	–	60% (12/20)	15% (3/20)
Healthy enamel with PFV 21 days	15% (3/20)	–	65% (13/20)	20% (4/20)
Healthy enamel with PFV 21 days polished	25% (5/20)	–	70% (14/20)	5% (1/20)
Demineralized enamel unvarnished	60% (12/20)	–	35% (7/20)	5% (1/20)
Demineralized enamel with CWV 24 h	25% (5/20)	–	65% (13/20)	10% (2/20)
Demineralized enamel with CWV 24 h polished	65% (13/20)	–	20% (4/20)	15% (3/20)
Demineralized enamel with CWV 21 days	–	–	85% (17/20)	15% (3/20)
Demineralized enamel with CWV 21 days polished	10% (2/20)	10% (2/20)	60% (12/20)	20% (4/20)
Demineralized enamel with PFV 24 h	75% (15/20)	–	25% (5/20)	–
Demineralized enamel with PFV 24 h polished	25% (5/20)	–	60% (12/20)	15% (3/20)
Demineralized enamel with PFV 21 days	45% (9/20)	–	40% (8/20)	15% (3/20)
Demineralized enamel with PFV 21 days polished	40% (8/20)	–	55% (11/20)	5% (1/20)

CWV: Clinpro White Varnish; PFV: Profluorid Varnish.

CWV, analysis of the association between polishing and the type of fracture for each type of varnish showed an association between polished varnish and cohesive enamel fractures and between unpolished varnish and adhesive fractures ($X^2 = 10.422$; $p = 0.015$; power = 0.783). However, polishing and the type of fracture for PFV were not related ($X^2 = 1.896$; $p = 0.387$; power = 0.205).

SEM Observations

SEM images showed healthy bovine enamel with typical marks caused by food abrasion (Fig 2a). Demineralized enamel (Fig 2b) had a very porous appearance, typical of acid attack. Healthy demineralized enamel varnished with CWV (Fig 3) or PFV (Fig 4) was covered with a homogeneous layer dotted with amorphous precipitates, both at 24 h and 21 days. When the varnish was removed by polishing, the surface of the healthy enamel appeared intact and the surface of the demineralized enamel showed repaired porosities, with a greater degree of repair observed at 21 days.

DISCUSSION

This study evaluated the effect of CWV, a varnish containing sodium fluoride (1%-5%) and functionalized tricalcium phosphate (<5%), and PFV, a varnish containing sodium fluoride (2.5%-5%), on the SBS of the Futurabond M+ universal adhesive on healthy and demineralized bovine enamel.

The results show that Futurabond M+ plus GrandioSO had a significantly higher SBS to healthy enamel than to demineralized enamel. The deficient infiltration of resin due to the intrinsic humidity of porous enamel, together with the dilution of water-soluble resin monomers, resulted in a lack of resin tags that could otherwise provide adequate micromechanical retention.[2,12] Other studies have suggested that additional factors are involved, such as the instability of the adhesive interface, which could contain a greater amount of protein, and microfiltration around the edges of the restoration, because these outlying areas have a lower concentration of minerals and a higher amount of protein.[4] In fact, one study[6] showed that deproteinization with a 12%

aqueous solution of chlorine dioxide (ClO_2) of the enamel surface of primary teeth affected by hypocalcified amelogenesis imperfecta, characterized by hypomineralized enamel with a high protein content, produced a significant increase in SBS, while the same treatment of healthy enamel had no effect on adhesion.[6]

Although the shear bond strengths obtained to demineralized enamel were lower than that to healthy enamel, the values were still above those accepted as clinically sufficient (17-24 MPa) to withstand the forces generated by polymerization contraction and ensure satisfactory retention of resin composite restorations bonded to teeth in function, both anterior and posterior.[33] It is believed that the composition of the adhesive used could be the reason, since the Futurabond M+ universal adhesive, which can be used with the etch-and-rinse technique or with a self-etching technique, contains alcohol, allowing it to react with and displace water molecules. It also contains the hydrophilic monomer 2-hydroxyethyl methacrylate (HEMA) and other acidic adhesive monomers that form a chemical bond with hydroxyapatite, which facilitates adhesion, together with the micromechanical bond.[37]

The two varnishes used in this in vitro study on bovine enamel behaved differently, and thus the null hypothesis was rejected. While CWV yielded a reduction in SBS to healthy and demineralized enamel at 24 h and 21 days, which recovered when the varnish was removed by polishing, PFV yielded an increase in SBS at 24 h and 21 days to both healthy and demineralized enamel. The increase was greater than the values in the unvarnished groups, independent of the surface treatment performed. This may have been due to the differing composition and physical characteristics of the products, especially the differing viscosities. Shen et al[29] found that CWV is more viscous than PFV and, due to its greater viscosity, CWV could be exerting a barrier effect (Fig 5). Thus, when the varnish was removed by polishing, there was a significant increase in SBS, which was similar at 24 h and 21 days in demineralized and healthy enamel surfaces. The interference by CWV with bonding is also reflected in the type of fracture, as there was an association between adhesive fractures and unpolished varnish, as well as between cohesive enamel fractures and polished varnish.

The presence of alcohol as a solvent and of low molecular weight monomers facilitates high penetration of Futurabond M+ adhesive into porous demineralized enamel. We suggest that this is the reason for both the high SBS to demineralized enamel and the significant association between cohesive enamel fractures and demineralized enamel. These values may reflect the cohesive force of enamel with a weakened structure,[20] rather than the adhesive force of Futurabond M+, which would be much greater. In fact, in the more resistant healthy enamel, the types of fracture observed were adhesive and, when the force was very high, cohesive fractures of the resin composite itself.

In some studies, adhesives with lower adhesive forces show a predominantly adhesive fracture pattern, while cohesive and mixed patterns occur in adhesives with higher adhesive forces.[17] Perdigão et al[26] found that when the adhesive force was greater than 17.40 MPa, cohesive fractures begin to appear. According to other authors, the fracture pattern is not related to the bonding force.[23]

Significant associations were found between cohesive and mixed fractures and the 24-h application timepoint, between adhesive fractures and the 21-day timepoint. This may be due to the disappearance of the microporosities of the demineralized enamel due to the repair of hydroxyapatite crystals and the formation of CaF_2 globules or other fluoride compounds that would reduce enamel permeability.[10] In fact, Choi et al,[11] using SEM, found that pretreatment with acidulated phosphate fluoride (APF) gel decreased microporosity formation in enamel in both the primary and permanent dentition from 10 days onwards.

Some resistance of the enamel to acid after topical application of fluoride might be another cause of the predominance of adhesive fractures over time,[18,32] as fluoride would gradually be incorporated into dental tissue to create an acid-resistant layer on the enamel surface, creating a chemical barrier that could interfere with the ability of phosphoric acid to effectively etch the enamel surfaces.[5] Varnish residues between the enamel prisms (Fig 6) could also act as a physical barrier, isolating the prism, and preventing further penetration of the acid and adhesive on the enamel surface.

No clear explanation for the increase in SBS with PFV at 21 days to healthy enamel and at 24 h and 21 days to demineralized enamel can be offered. Although varnish residues are observed on the enamel surface in SEM images, even after polishing, this is not reflected in the SBS, as was the case with the CWV. We thought that PFV, which has a lower viscosity than CWV, might be dislodged by rubbing-on Futurabond M+, and hence not interfere with enamel adhesion. To the present authors' knowledge, no study evaluating the influence of PVF on bonding to enamel has been published to date. Studies[22] have found an increase in bond strength using 1.23% APF on bovine enamel and suggested this was due to the characteristics of the substrate. However, other studies using bovine enamel with fluoride varnishes other than PFV found no effect or a reduction in bond strength according to the type of adhesive used.[8,14]

The present study has other limitations. Although human tooth is the ideal substrate to test bonding to enamel, bovine teeth were used because it is difficult to obtain sufficient numbers of human incisors with adequate quality and with flat labial surfaces large enough to perform the SBS test. Even though there are histological differences, bovine teeth are a reliable substitute for human teeth in bond strength studies of adhesion to both enamel and dentin substrates.[31] Although some authors consider the SBS test to be more representative of the clinical situation,[24] its results are influenced by various parameters, including substrate type, material, bonding area and test design/execution. A strong correlation was found between higher bond strength and the proportion of cohesive failures.[35] This should be borne in mind when interpreting the results.

CONCLUSION

The present in vitro results show that the remineralization of demineralized enamel using fluoride varnishes permits recovery of the bond strengths obtained to healthy enamel. Of the two varnishes studied, PFV yielded the better results, achieving higher SBS and more uniform behavior, regardless of the varnish application timepoint, enamel surface condition, and polishing of the enamel. Remineralization of demineralized enamel reduced the number of cohesive fractures.

In clinical situations where the enamel has been previously treated with fluoride varnish, polishing of the surface before the restorative procedures is recommended to eliminate any interference of varnish remnants and to ensure the bonding performance of dental adhesives.

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Clinical relevance: Enamel remineralized with a varnish containing sodium fluoride may improve the adhesion capacity/strength of a universal adhesive.