Bonding Universal Dental Adhesive to Developmentally Hypomineralised Enamel

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Purpose: To investigate the effect of pretreatment protocols involving Papacarie Duo gel and Scotchbond Universal (SU) on the microshear bond strength (μSBS) of resin composite (RC) to hypomineralised enamel (HE).

Materials and Methods: Specimens of normal enamel (NE) and HE were derived from extracted hypomineralised first permanent molars (FPMs). Based on the colour of demarcated opacities, HE specimens were classified as creamy/white (CW) or yellow/brown (YB). The specimens were randomly allocated into eight groups (n = 20). Each group involved pretreatment with Papacarie Duo gel or no pretreatment, and SU applied in etch-and-rinse (E&R) or self-etch (SE) mode. All specimens were bonded with RC and subjected to μSBS testing. Failure modes were analysed using an optical microscope and SEM.

Results: Comparing NE with HE, the following factors were found to be significant ($p < 0.001$): type of enamel substrate, deproteinising pretreatment, and etching mode. Comparing CW HE with YB HE, a significant interaction between "deproteinising pretreatment" and "etching mode" was demonstrated ($p = 0.028$). When subjected to the concurrent use of Papacarie Duo gel and phosphoric acid etching, HE specimens showed a significant increase in μSBS ($p < 0.001$).

Conclusion: Deproteinising pretreatment using Papacarie Duo gel followed by the application of SU in E&R mode led to increased μSBS of resin composite to HE.

Keywords: bonding, deproteinisation, hypomineralised enamel, microshear bond strength, Papacarie, Scotchbond Universal.

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Molar Incisor Hypomineralisation (MIH) is a developmental dental defect of systemic origin, which invariably affects one or more first permanent molars (FPMs) with or without involvement of the permanent incisors.[53] It describes the clinical appearance of demarcated opacities, modified translucency, and qualitative dilapidations within enamel.[17,34] Similar aberrations may also occur in other teeth, commonly primary canines, second primary molars, second permanent molars and the cuspal tips of permanent canines.[13,16,22,37] With a pooled mean prevalence of 14.2% worldwide, MIH affects approximately one in seven children.[57]

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Hypomineralised enamel (HE) is associated with a decrease of 19–20% in mineral density and a significant 8- to 21-fold increase in protein content.[17,20,34] These anomalous changes to the chemomechanical properties of HE severely undermine the ability to withstand dynamic masticatory forces, parafunctional bite forces and other insults within the oral environment. As a consequence, MIH-affected teeth are at risk of hypersensitivity, post-eruptive breakdown and secondary caries.[52]

Despite considerable research efforts, the restorative management protocol of MIH remains indeterminate.[31,33,55] Restorative treatment outcomes are often unpredictable, thus subjecting affected individuals to a tumultuous cycle of retreatments and a great economic burden at high sociopsychological costs.[27] By the age of 9 years, children with severe MIH are reported to be almost 10 times more likely to have received dental treatment on one or more of their hypomineralised FPMs than their non-affected peers.[28] These findings profoundly highlight the limitations of MIH when bonding dental adhesive materials. Multiple studies have indicated that adhesion to HE is achievable, but with varying degrees of success.[32,34] The bond strength of resin composite to HE has been shown to decrease by 25%–60%.[8,14,54] In an attempt to address these bonding constraints, the integration of a pretreatment protocol to facilitate the removal of intrinsic proteins encasing hydroxyapatite has been propounded.[32,33]

Several studies investigating the effect of various deproteinising agents, including sodium hypochlorite (NaOCl) and Papacarie Duo gel, on NE and HE have emerged with promising results.[3,14,42,43] In particular, pretreatment with Papacarie Duo gel is associated with enhanced enamel bond strength without the risks and complications associated with the use of NaOCl.[3,14,25,42,43] Although its precise mechanism of action has yet to be fully elucidated, its main action is primarily driven by papain, which is a proteinase known to have anti-inflammatory, bacteriostatic and bactericidal properties.[6,24,25] To date, only one published in vitro study has investigated the effect of Papacarie Duo gel on HE, and the positive results indicate it is an efficacious deproteinising agent.[14]

Over the past decades, the development of new dental adhesives have challenged the principles of restorative dentistry.[47] Adopting the "all-in-one" approach, these products are designated as "multi-mode" or "multi-purpose" because they can be used in three different etching modes when bonding to tooth substrate: self-etch (SE), etch-and-rinse (E&R), or selective enamel etch.[9,40,47] Contrary to the older generation of dental adhesives, the composition of universal dental adhesives is unique.[45,47] This is due to the inclusion of acidic hydrophilic monomers such as specific carboxylate and/or phosphate monomers, eg, 10-methacryloyloxydecyl dihydrogen phosphate (10-MDP), which act as primers while promoting ionic bonding to calcium in hydroxyapatite.[47]

Currently, there is a dearth of data on the efficacy and effectiveness of universal dental adhesives in association with developmental dental defects. Thus far, there has only been one in vitro study investigating the microtensile bond strength of resin composite to HE using Scotchbond Universal (SU) (3M Oral Care; St Paul, MN, USA) in E&R mode, and the authors have observed significantly lower bond strength values among HE specimens.[29] Moreover, a recently published in vivo study on MIH has become the first randomised controlled trial to investigate the survival rate of resin composite restorations bonded with a universal dental adhesive (Ambar Universal APS; FGM, Brazil).[44] Although E&R mode was associated with a higher retention rate (80.8%) than SE mode (62.3%) at 12 months after treatment, the difference was not statistically significant.[44] It is important to recognize that neither study included a deproteinising pretreatment in their investigation of universal dental adhesives on MIH-affected teeth.

The aim of the present study was to investigate the effect of various pretreatment protocols involving Papacarie Duo gel and SU on the microshear bond strength (μSBS) of resin composite to HE. The following hypotheses were tested: 1. there would be a difference in enamel bond strength between NE and HE specimens when subjected to the same protocol; 2. pretreatment of NE and HE specimens with deproteinising Papacarie Duo gel would increase the bond strength of resin composite to enamel when conditioned with the same etching mode; and 3. there would be a difference in enamel bond strength between E&R mode and SE mode when conditioned on the same type of enamel substrate and subjected to the same protocol.

MATERIALS AND METHODS

Ethics

The present study was initially reviewed by the Health and Disability Ethics Committees (New Zealand Ministry of Health), and Māori consultation was conducted with the Ngāi Tahu Research Consultation Committee. Ethical approval was obtained for the collection and experimental use of extracted teeth from the Human Ethics Committee (Health) at the University of Otago (H18/086). To facilitate the collection of extracted teeth from other regions in New Zealand, locality authorisation and further Māori consultation were also sought from various district health boards and their affiliated research committees.

Collection of Teeth

Over a 13-month period, a pooled sample of 95 erupted hypomineralised first permanent molars (FPMs) were extracted and collected from participants between the ages of 6 and 18 years inclusively, with an established diagnosis of MIH. To ensure the validity of the study, participants with an underlying medical condition and/or identified syndrome that may potentially be associated with the development of dental defects were excluded.

All participants were under the care of paediatric dentists in either private specialist practices or public hospitals within New Zealand. Following the standard of care and as part of a personalised treatment plan, hypomineralised

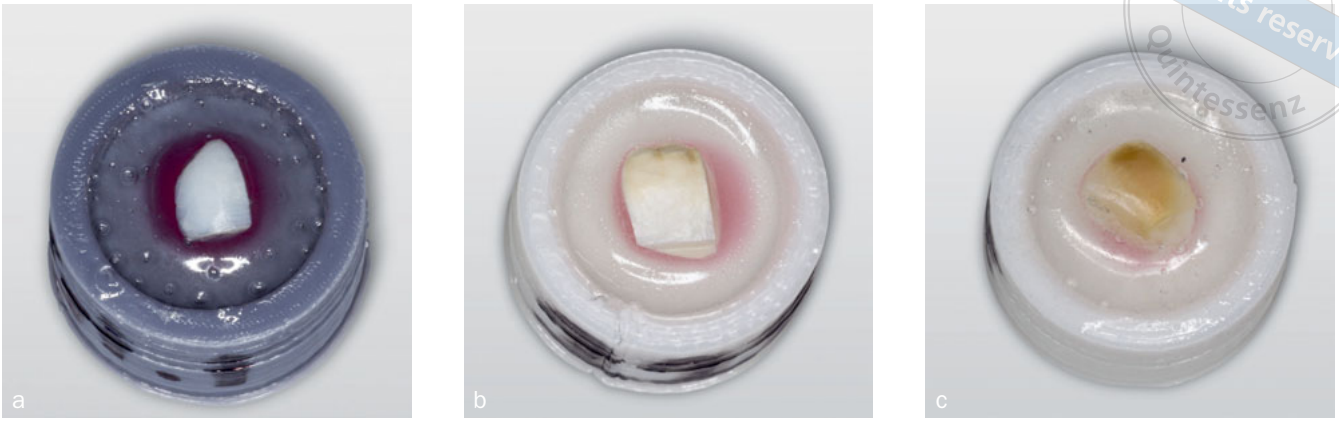


Fig 1 Photographs of the archetypal specimens that were set apart and used as reference points in the identification of enamel substrates. (a) normal enamel (NE); (b) creamy/white (CW) hypomineralized enamel (HE); (c) yellow/brown (YB).

FPMs were extracted due to post-eruptive breakdown, associated secondary caries or for orthodontic purposes, and it was common to have more than one FPM extracted per participant. Prior to the collection of teeth, age-appropriate information sheets outlining the research project were also provided to the participant and parent/legal guardian, and both parties were required to give written assent and informed consent, respectively.

Participating clinics were supplied with a storage-related instruction sheet and specimen bottles that contained 0.10 g of thymol crystals (Sigma-Aldrich; St Louis, MO, USA), and temporary storage did not exceed four weeks. Upon receipt, the extracted teeth were deidentified and cleaned using a slow-speed fine rotary brush and pumice to remove adherent residues of plaque, blood, soft tissue and other contaminants.

Adopting the MIH judgment criteria established by Weerheijm et al,[51] the teeth were wet and visually inspected under daylight conditions by the investigator to confirm the clinical diagnosis of MIH, and verify the availability of at least 2–3 mm diameter of NE and/or HE in the occlusal half of the tooth. The teeth were then transferred to clean specimen bottles containing 0.5% thymol solution compounded by 20 ml of Milli-Q distilled deionised water (DDW) and 0.10 g of thymol crystals, and they were stored in a controlled environment at 4°C until use.

Preparation of Specimens

The roots of hypomineralised FPMs were embedded in cold-setting EpoFix resin (Struers; Copenhagen, Denmark) cylinders. Clamping on the resin embedment for anchorage, the extracted teeth were individually mounted on an automated precision cutting machine (Accutom-50, Struers; Copenhagen, Denmark), and the crowns were sectioned using a 0.4-mm-thick diamond-impregnated disk (M1D13; Struers) under water cooling.

The enamel specimens were derived from the occlusal half of coronal structure to ensure the thickness of all specimens was consistent and adequate. To reduce the risk of bias, specimens for NE were also acquired from the same sample group of extracted hypomineralised FPMs. In most cases, at least two NE specimens and up to four HE specimens could be obtained per extracted tooth. None of the specimens were re-used in the experiment.

The enamel specimens were manually, minimally polished with wet P1200-grit silicon carbide paper (Riken Corundum; Saitama, Japan) to remove the outermost layer of aprismatic enamel (approximately 30 µm thick). This process aimed to conserve as much enamel tissue as possible, and the specimens were uniformly subjected to a fixed number of polishing strokes. All specimens were subsequently embedded in 3D-printed cylindrical resin moulds using cold-setting EpoFix resin, and each of them was assigned an arbitrary number.

Identification of NE and HE substrates

In addition to the initial inspection conducted immediately after cleaning, a second visual assessment of enamel specimens was independently performed by two of the present authors (YL and ME). The objective of the procedure was to validate the accuracy of the initial visual inspection. Likewise, the second inspection used the MIH judgment criteria[51] and was carried out under the same examination conditions. Prior to the assessment, the specimens were left at room temperature for 24 h in hermetic containers containing Milli-Q DDW.

A few archetypal examples of NE, creamy/white (CW) HE, and yellow/brown (YB) HE specimens were set apart and used as reference points for differentiating enamel substrates (Fig 1). NE was defined as “normal-looking dental enamel tissue”, devoid of delineated opacities or surface discolouration, developmental defects, and caries. Because considerably less mineralised prism sheaths and lower mechanical properties in the transitional area immediately adjacent to the demarcated borders of HE have been reported,[7] NE specimens in this study were carefully obtained in regions some distance from HE. In contrast, HE referred to visual defects with altered translucency and demarcated

Table 1 Materials used in the study

Material	Main constituents	pH	Manufacturer
Scotchbond Multi-Purpose Etchant	37% phosphoric acid, polyvinyl alcohol, water	0.1	3M Oral Care; St Paul, MN, USA
Scotchbond Universal	10-MDP, HEMA, dimethacrylate resins, Vitrebond copolymer (acryl- and itacon acid), filler, ethanol, water, silane, photoinitiators (ultra-mild universal dental adhesive)	2.7	3M Oral Care
Papacarie Duo	Papain, chloramine, toluidine blue, preservatives, salts, stabilizers, thickener, deionised water	9.2[24]	Formula & Acao; SP, Brazil
Filtek Supreme XTE Universal Restorative (resin composite)	Bis-GMA, UDMA, bis-EMA(6), TEG-DMA, PEG-DMA, silane-treated ceramic, silica filler, zirconia filler, zirconia/silica cluster filler, and others (shade: A2B)		3M Oral Care

Bis-EMA: bisphenol A polyethylene glycol diether dimethacrylate; bis-GMA: 2,2-bis [4-(2-hydroxy-3-methacryloyloxypropoxy) phenyl] propane; HEMA: 2-hydroxyethyl methacrylate; 10-MDP: 10-methacryloyloxydecyl dihydrogen phosphate; PEG-DMA: polyethylene glycol dimethacrylate; TEG-DMA: triethyleneglycol dimethacrylate; UDMA: urethane dimethacrylate.

Table 2 Outline of pretreatment and bonding protocols

SE mode						E&R mode (PA etching)					
No deproteinisation			Deproteinisation			No deproteinisation			Deproteinisation		
Group 1 NE	Group 5W CW HE	Group 5Y YB HE	Group 3 NE	Group 7W CW HE	Group 7Y YB HE	Group 2 NE	Group 6W CW HE	Group 6Y YB HE	Group 4 NE	Group 8W CW HE	Group 8Y YB HE
			Papacarie gel actively applied with agitation (60 s)						Papacarie gel actively applied with agitation (60 s)		
			Water spray (60 s)						Water spray (60 s)		
			Air dried (15 s)						Air dried (15 s)		
						37% PA etch (15 s)			37% PA etch (15 s)		
						Water spray (30 s)			Water spray (30 s)		
SU: single layer applied to air-dried enamel surface in rubbing motion (20 s)			SU: single layer applied to air-dried enamel surface in rubbing motion (20 s)			SU: single layer applied to air-dried enamel surface in rubbing motion (20 s)			SU: single layer applied to air-dried enamel surface in rubbing motion (20 s)		
Air-thinned (5 s)			Air thinned (5 s)			Air-thinned (5 s)			Air thinned (5 s)		
Light curing (10 s)			Light curing (10 s)			Light curing (10 s)			Light curing (10 s)		
Brass tube stabilised on specimen			Brass tube stabilised on specimen			Brass tube stabilised on specimen			Brass tube stabilised on specimen		
RC packed in 2 increments of 1 mm; each increment light cured for 40 s			RC packed in 2 increments of 1 mm; each increment light cured for 40 s			RC packed in 2 increments of 1 mm; each increment light cured for 40 s			RC packed in 2 increments of 1 mm; each increment light cured for 40 s		

PA, phosphoric acid; Papacarie, Papacarie Duo gel; RC, resin composite; SU, Scotchbond Universal.

opacities within dental enamel. Based on the colour of the demarcated opacities to determine the severity of hypomineralisation and the degree of porosity, HE specimens were further classified into CW and YB.

Power Calculation of Sample Size

Based on previous MIH in vitro studies with similar aims and designs, the sample size was calculated to be 20 enamel specimens per experimental group, with an equal number of 10 CW HE and 10 YB HE specimens allocated to each HE group.[8,14,54]

The design of this study was designated to be A x B x C with 2 x 2 x 2 factor levels, yielding a total of 8 experimental groups. Using G*POWER statistical software (version 3.1.9.3, Jochen Grommisch; Aichach, Germany) to compute a priori power analysis, a minimum sample size of 128 enamel specimens was required to acquire a recommended power of 0.80 in testing three-factor interaction at the statistical significance level of $\alpha = 0.05$.[5,21] However, to ensure equal group sizes and to account for possible pre-test failures, a total sample size of 160 enamel specimens was determined. A post-hoc power analysis revealed that the power of the present study was increased to 0.88.

Randomised Allocation of Specimens

All enamel specimens were randomised on Microsoft Excel (Microsoft; Redmond, WA, USA) and distributed into eight experimental groups, with groups 1 and 2 being designated

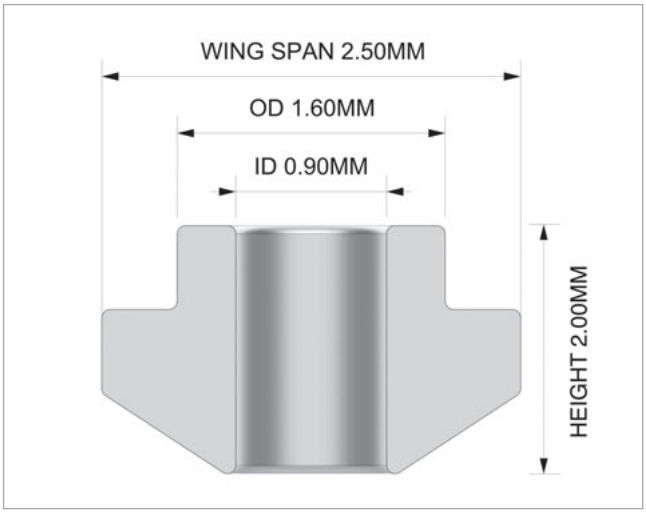
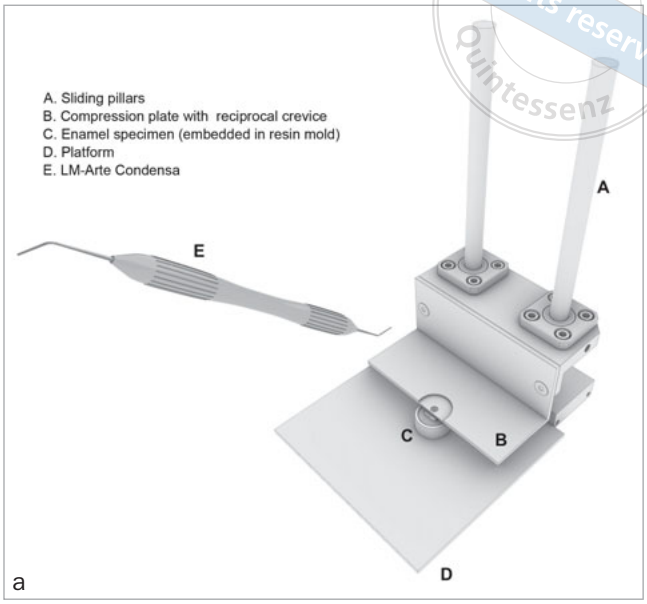


Fig 2 Diagram of a cylindrical brass tube with bevelled base. The shaded area represents the hollow space. Dimensions are as labelled. OD: outer diameter; ID: inner diameter.



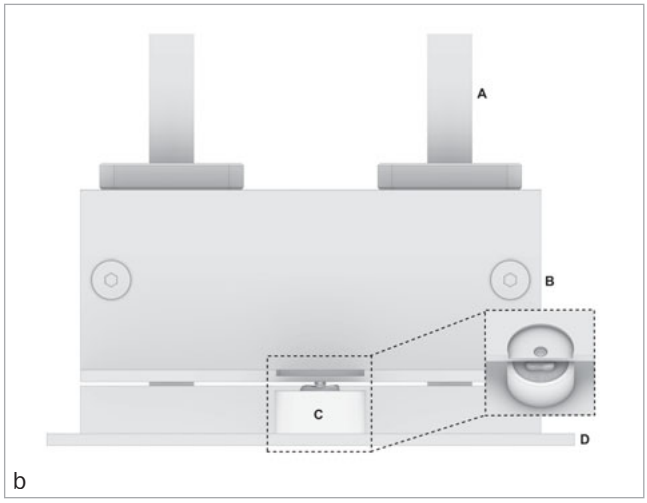


Fig 3 Schematic diagrams of custom-made apparatus and bonded assembly. (a) Extra-fine restorative instrument (E) was used to pack resin composite into the hollow brass tube; (b) brass tube is firmly stabilised by the reciprocal crevice, with the compression plate sitting on the circumferential wing of the brass tube.

as control groups. HE groups were also divided into subgroups of CW and YB, which were denoted by the suffixes -W and -Y, respectively.

Pretreatment and Bonding Protocol

The materials used in this study are shown in Table 1. Protocols for the eight experimental group are further described in Table 2.

In groups 3, 4, 7 and 8, the enamel specimens were first deproteinised with papain-based Papacarie Duo gel (Formula & Acao; SP, Brazil) via active application for 60 s. This was followed by rinsing with a copious amount of water from a three-way dental syringe for 60 s, and thorough drying using oil-free air for 15 s.

Enamel specimens in E&R groups 2, 4, 6 and 8 were treated with 37% phosphoric acid etchant (Scotchbond Multi-Purpose Etchant, 3M Oral Care) for 15 s. Using the three-way dental syringe, etched surfaces were vigorously rinsed with water for 30 s and dried with oil-free air for 15 s until a matte white or frosted appearance was clinically visible. On the other hand, enamel specimens in SE groups 1, 3, 5 and 7 were not subjected to phosphoric acid etching.

Following manufacturer's instructions, a single layer of SU was actively applied on all specimens in a rubbing motion for 20 s and air dried for 5 s to allow the solvent to evaporate. The adhesive was photopolymerized for 10 s using a light-emitting diode (LED) curing light (VALO corded curing light, Ultradent; South Jordan, UT, USA) with an emittance of 900 mW/cm^2 and at a distance of 1 mm.

A custom-milled, hollow cylindrical brass tube (Fig 2) with an internal diameter of 0.9 mm and a height of 2.0 mm was placed on each enamel specimen and firmly secured by a reciprocal crevice that was unique to the compression plate of a custom-made apparatus (Fig 3).[14] The dimensions of the reciprocal crevice correlated with those of the brass tube to ensure good stabilisation. Using an extra-fine restorative instrument (LM-Arte Condensa, LM-Dental; Pargas/Parainen, Finland) to pack, the hollow brass tubes were filled with resin composite (Filtek Supreme XTE Universal Restorative, 3M Oral Care). As the brass tubes were opaque, resin composite was packed in two separate increments of 1.0 mm, with each incremental layer being subjected to 40 s of light curing (within a distance of 2 mm) to eliminate the risk of inadequate polymerisation.

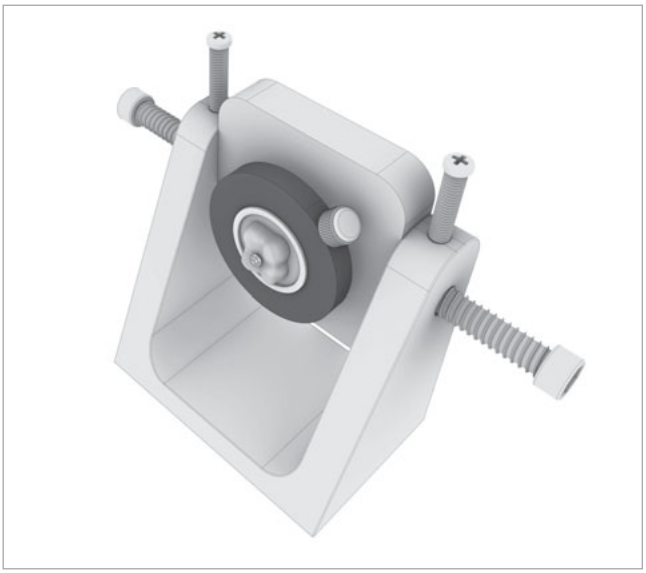


Fig 4 The 3D-printed resin mounting jig in which bonded enamel specimens were firmly secured prior to μSBS testing. The dark grey circular attachment is non-fixated and self-manoeuvrable, which helps to ensure precise delivery of microshear force to the resin-enamel adhesive interface.

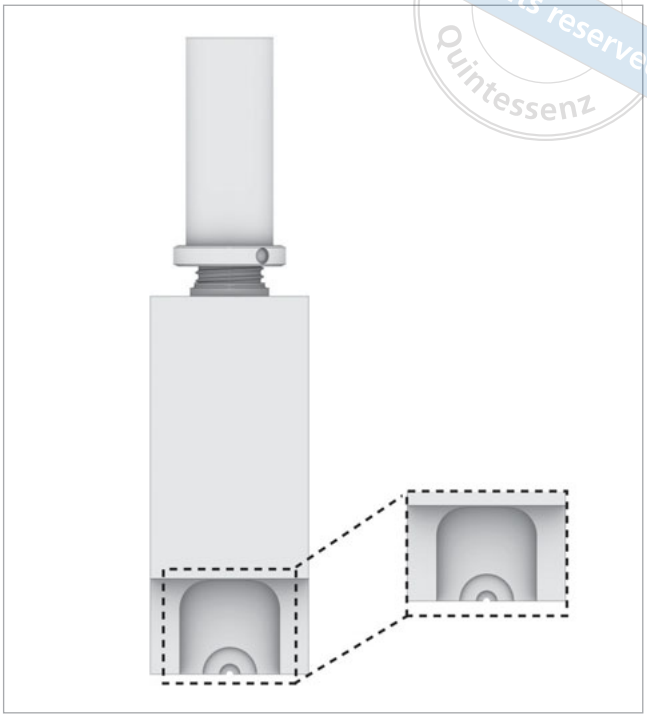


Fig 5 Customised stainless steel shearing fixture with novel lip configuration. The lip has a thickness of less than 0.1 mm, and its dimensions correspond with the base of the brass tube.

Microshear Bond Strength (μSBS) Test

The bonded enamel specimens were incubated at 37°C in Milli-Q DDW for 24 h before μSBS testing. Prior removal of the brass tubes was not required due to their bevelled base and the precision engineering behind their seating in relation to the enamel surface. Specimens were secured horizontally on a 3D-printed resin mounting jig (Fig 4), and they were loaded to failure at a crosshead speed of 1.0 mm/min using a customised stainless steel shearing fixture (Figs 5 and 6) that was attached to a universal testing machine (Model 3369, Instron; Canton, MA, USA). Subsequently, μSBSs (MPa) were calculated as the peak loading force needed to shear the resin composite-filled cylindrical brass tube divided by the bonded surface area.

Failure Mode Analysis

After the μSBS test, debonded enamel surfaces and resin composite-filled cylindrical brass tubes were examined under an optical microscope (SMZ800N, Nikon; Tokyo, Japan) at 30–40X magnification. Digital images were obtained and failure modes were categorised as follows: 1. adhesive failure: the plane of failure was along the resin-adhesive–enamel interface; 2. cohesive failure in resin composite: the debonded substrate surface showed fracture through resin composite; 3. cohesive failure in enamel: the debonded substrate showed fracture through dental enamel; 4. mixed failure: when two or more modes of fail-

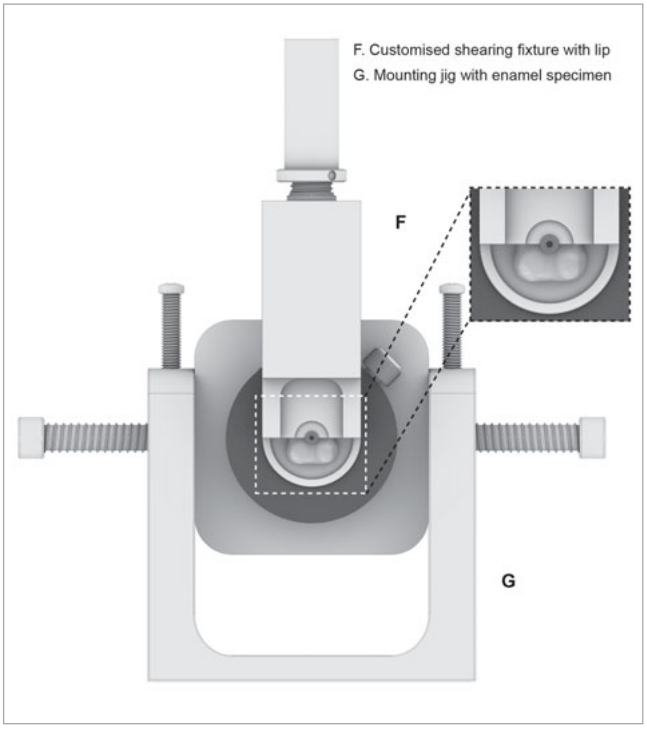


Fig 6 Schematic diagram to illustrate the precise engagement of the lip with the resin composite-filled cylindrical brass tube at the bonded interface during μSBS testing.

Table 3 Mean microshear bond strength of resin composite to normal enamel and hypomineralised enamel

	Mean μSBS (MPa) ± SD			
(n = 20)	SE mode	E&R mode	Deproteinisation & SE mode	Deproteinisation & E&R mode
Normal enamel	6.2 ± 2.7^{aA}	8.9 ± 2.8^{cB}	7.3 ± 2.6^{eAB}	10.4 ± 2.7^{gC}
Hypomineralised enamel (CW and YB)	3.5 ± 2.0^{bD}	5.6 ± 2.5^{dE}	4.3 ± 1.9^{fDE}	8.8 ± 1.9^{hF}

CW: creamy/white; YB: yellow/brown. E&R: etch-and-rinse; μSBS: microshear bond strength; SD: standard deviation; SE: self-etch. Values with the same superscript lowercase letters in columns indicate no significant difference at α = 0.05. Values with the same superscript uppercase letters in rows indicate no difference at a significance level of α = 0.05.

ure were present. If a specific mode of failure was identified in 80% or more of the debonded substrate area, it was deemed predominant and classified as such. When less than 80% of the debonded substrate area exhibited a particular failure mode, it was categorised as a mixed failure.

Representative enamel specimens from each failure mode were selected for qualitative evaluation with scanning electron microscopy (SEM) (Zeiss Sigma, Carl Zeiss; Oberkochen, Germany). Prior to the observation, samples were left at room temperature to air dry for 24 h. The enamel surfaces of the specimens were coated with 10 nm of gold palladium in a sputter-coating vacuum (Q150T, Quorum Technologies; Lewes, East Sussex, UK), and mounted onto aluminium stubs using carbon tape and carbon paint. The specimens were qualitatively examined under magnifications up to 2000X, at an acceleration voltage of 10 kV.

Statistical Analysis

All statistical analyses were performed using SPSS software (version 26.0.0.1, IBM; Armonk, NY, USA), with the level of statistical significance set at $\alpha = 0.05$. The μSBS data for each experimental group were tested for homogeneity of variance using Levene's test. Three-way ANOVA followed by post-hoc hypothesis testing was also used to analyse the μSBS data, as well as to examine the effect and interaction of the following three factors: 1. type of enamel substrate; 2. etching mode; and 3. deproteinising pretreatment.

Subsets of μSBS data pertaining to HE groups 5 to 8 were further analysed in accordance with the severity of enamel hypomineralisation. Similarly, three-way ANOVA and post-hoc hypothesis testing were used to ascertain the effect and interaction of the following three factors: 1. severity of hypomineralisation; 2. etching mode; 3. deproteinising pretreatment.

RESULTS

Microshear Bond Strength (μSBS)

NE and HE

The means and distribution of μSBS values (MPa ± SD) for NE and HE specimens are presented in Table 3. With a p-value of 0.189 derived from Levene's test for equality of variances, the homogeneity of the μSBS data for each experimental group was validated. Three-way ANOVA suggested that each of the following three factors were significant ($p < 0.001$): 1. type of enamel substrate; 2. etching mode; 3. deproteinising pretreatment. However, none of the interactions between the factors were significant.

Comparing NE with HE specimens, there was a statistically significant difference in mean μSBS values when subjected to the same pretreatment protocol. Although there was a significant difference between groups 4 and 8 ($p = 0.030$), it was apparent that pretreatment of HE with Papacarie Duo gel followed by phosphoric acid etching led to an estimated increase of 20% in bond strength. It was also found that the mean μSBS for group 8 closely approximated that of group 2. This inferred that the bond strength of deproteinised acid-etched HE specimens was comparable to that of non-deproteinised acid-etched NE specimens.

Collectively analysing HE specimens as a group, it was evident that the deproteinising pretreatment did not exert a significant effect on bond strength when it was used in conjunction with SU in SE mode ($p = 0.292$). In comparison, HE specimens from group 8 exhibited the greatest increase in mean μSBS (8.76 ± 1.89 MPa), indicating that the integration of Papacarie Duo gel as a pretreatment prior to E&R mode had a significant impact on enamel bond strength ($p < 0.001$). These findings were also consistently observed among NE specimens, with a statistically significant difference noted between groups 2 and 4 ($p = 0.04$).

CW HE and YB HE

The means and distribution of μSBSs (MPa ± SD) for CW HE and YB HE specimens are summarised in Table 4. Three-way ANOVA indicated that each of the following three factors were significant ($p < 0.001$): 1. severity of hypomineralisation: 2. etching mode, and 3. deproteinising pretreatment. A significant interaction between "etching mode" and "deproteinising pretreatment" was also identified ($p = 0.028$). Investigating this interaction via Simple Main Effects analysis, it was shown that "deproteinising pretreatment" significantly increased enamel bond strength only when it was supplemented with phosphoric acid etching in E&R mode.

Table 4 Mean microshear bond strength of resin composite to hypomineralised enamel of different severity

	Mean μSBS (MPa) ± SD			
(n = 10)	SE mode	E&R mode	Deproteinisation & SE mode	Deproteinisation & E&R mode
Creamy/white hypomineralised enamel	4.4 ± 2.0^{aA}	7.8 ± 2.5^{cB}	4.9 ± 1.6^{eA}	9.2 ± 2.1^{fB}
Yellow/brown hypomineralised enamel	2.5 ± 1.5^{bC}	4.3 ± 1.6^{dD}	3.7 ± 2.1^{eCD}	8.3 ± 1.7^{fE}

E&R: etch-and-rinse; μSBS: microshear bond strength; SD: standard deviation; SE, self-etch. Values with the same lowercase letter in columns indicate no difference at a significance level of α = 0.05. Values with the same uppercase letter in rows indicate no difference at a significance level of α = 0.05.

Table 5 Distribution of failure modes across the eight experimental groups

	Control groups (n = 20)		Test groups (n = 20)					
	Group 1 NE SE mode	Group 2 NE E&R mode	Group 3 NE Deproteinisation & SE mode	Group 4 NE Deproteinisation & E&R mode	Group 5 HE SE mode	Group 6 HE E&R mode	Group 7 HE Deproteinisation & SE mode	Group 8 HE Deproteinisation & E&R mode
Mode of failure								
Adhesive	12 (60)	5 (25)	7 (35)	1 (5)	12 (60)	12 (60)	9 (45)	4 (20)
Cohesive in enamel	–	–	–	–	4 (20)	3 (15)	2 (10)	2 (10)
Cohesive in RC	–	2 (10)	2 (10)	5 (25)	–	–	1 (5)	1 (5)
Mixed	8 (40)	13 (65)	11 (55)	14 (70)	4 (20)	5 (25)	8 (40)	13 (65)

E&R: etch-and-rinse; HE: hypomineralised enamel; NE: normal enamel; RC: resin composite; SE: self-etch. Calculated percentage is represented in percent.

Comparing CW HE with YB HE specimens, there was no statistically significant difference in mean μSBS when they were subjected to deproteinisation followed by SE mode ($p = 0.167$) or E&R mode ($p = 0.327$). Analysing CW HE and YB HE specimens as two individual groups, it was further evident that Papacarie Duo gel did not have a significant effect on enamel bond strength when used in conjunction with SU in SE mode ($p = 0.604$ for CW HE; $p = 0.177$ for YB HE). These findings were consistent with those of NE specimens.

Failure Mode

The distribution of failure modes within each experimental group are presented in Table 5. The most common failure mode identified in NE was mixed failure, whereby partial adhesive failure and partial cohesive failure in resin composite were observed (57.5%), followed by adhesive failure (31.3%). A total of 11 specimens exhibited cohesive failure in enamel, all of which were severely HE substrates with demarcated YB opacities. In groups 7 and 8, deproteinisation of HE specimens was associated with a relatively lower occurrence of cohesive failure in enamel and an increase in mixed failure mode.

SEM Observation of Debonded Specimens

Representative SEM micrographs of adhesive-enamel interfaces pertaining to each failure mode are shown in Fig 7. Careful inspection of the interfaces revealed distinct differences in enamel surface morphology between E&R mode and SE mode. When subjected to phosphoric acid etching, NE specimens showed complete removal of smear layer, and uniform preferential dissolution of enamel prism cores and/or peripheral rods was evident (Figs 7b and 7c). This contrasted with acid-etched HE specimens in group 6, which had inherent cracks and showed irregular etching patterns (Fig 7f). However, CW HE and YB HE substrates in group 8 (Figs 7h and 7i) displayed more profound etching patterns and were associated with a marked increase in mixed failure modes when they were deproteinised before phosphoric acid etching.

Contrary to E&R mode, all enamel substrate types were found to have a hybridised smear layer when the surface was treated with SU in SE mode, irrespective of the use of Papacarie Duo gel. A higher prevalence of adhesive failure was detected amid NE specimens in group 1 (Fig 7a), with residual adhesive remaining on the debonded surface area. Furthermore, cohesive failure in enamel was most notable among HE specimens in group 5, whereby fractured enamel rods were evident (Fig 7d).

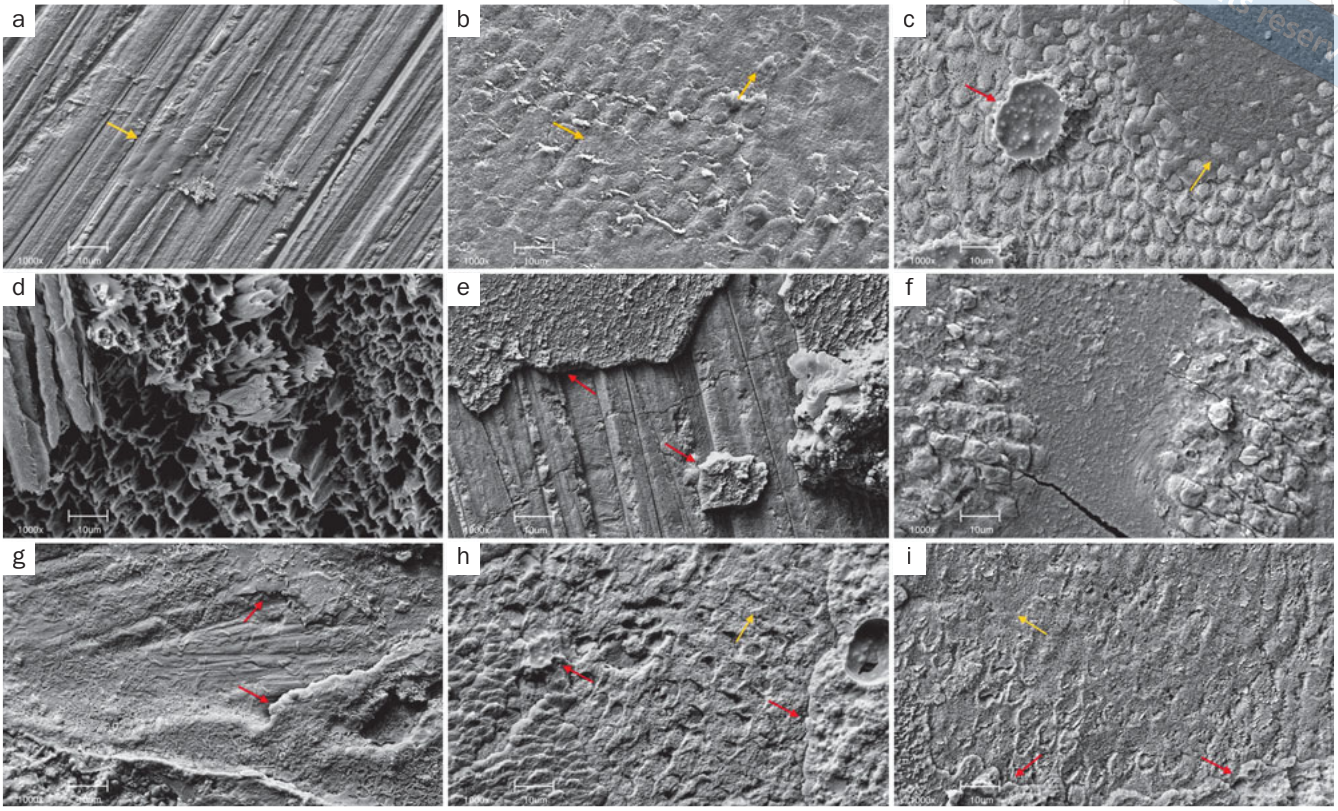


Fig 7 Representative SEM micrographs illustrating the adhesive-enamel interface of debonded specimens after μSBS testing. (a) Group 1 (NE): adhesive failure; (b) group 2 (NE): adhesive failure; (c) group 4 (NE): mixed failure with profound type 2 etching pattern; (d) group 5Y (YB HE): cohesive failure in enamel showing fractured enamel rods; (e) group 5W (CW HE): mixed failure; (f) group 6Y (YB HE): mixed failure with irregular etching pattern and inherent cracks; (g) group 7W (CW HE): mixed failure; (h) group 8W (CW HE): mixed failure with more defined etching pattern; (i) group 8Y (YB HE): mixed failure with more profound etching pattern. Annotations: yellow arrow indicates remnants of universal dental adhesive, and red arrow indicates resin composite.

DISCUSSION

Modified Methodology

The methodology of the present study was established upon that of a previous laboratory investigation by Ekambaram et al,[14] but with modifications to improve the experimental procedure as well as the performance of the μSBS test. The consistency of the testing geometry, standardisation of the apparatus and materials, and the simplicity of the μSBS test protocol not only allowed easy reproducibility, but also mitigated the risk of incorporating faults into the bonded assembly. As the experiment was completed by a single investigator, there were no concerns regarding inter-operator differences.

The reciprocal crevice, which was unique to the compression plate of the custom-made apparatus, firmly stabilised the brass tubes without needing to bond them to the enamel specimens (Fig 3). The bevelled base of the brass tubes (Fig 2) further helped to minimise the surface area with which the tubes were in contact. This obviated any potential distortional effect that might have been exerted by the brass tube on the μSBS test. As a result, no prior removal of brass tubes was necessary, and no pre-test failures were observed in this study.

Unlike past μSBS studies on dental adhesive materials, a customised stainless steel shearing fixture with a novel lip configuration was used to deliver the shear force (Fig 5). The lip was designed with a thickness of less than 0.1 mm, and its semi-circular shape and dimensions corresponded to the bevelled base of the cylindrical brass tubes. This enabled the shearing fixture to accurately engage with the base of the brass tube at the bonded interface. In addition, a 3D-printed resin mounting jig (Fig 4) was used to mount and clamp enamel specimens before μSBS testing. The constellation consisting of the mounting jig, brass tube, and the lip on the shearing fixture (Fig 6) allowed uniform distribution of the stress and ensured that the plane of the bonded interface was parallel to the vertical vector of the shear force.

Microshear Bond Strength (μSBS)

The μSBSs of resin composite to NE specimens were significantly higher than those of HE specimens when following the same pretreatment and bonding protocol. Therefore, the first hypothesis, that there would be a difference in enamel bond strength between NE and HE specimens, was supported. This is attributable to the different chemomechanical properties and aberrant crystalline structure of HE, which is comparatively porous and disorganised.[35]

Compared with NE, HE has an average mineral content of 59 vol%, which is approximately 27% lower than that of NE, and an estimated reduction of 50%–75% in microhardness and elastic modulus.[12,17] Reduced concentrations of calcium and phosphorus, and a substantial increase in carbon and carbonate contents have also been reported.[17,36] Qualitative evaluation of phosphoric acid-etched HE using SEM further revealed atypical etching patterns, poor intercrystal porosity, and non-uniform dissolution of enamel prism cores and/peripheral rods.[8,14,54] This caused exiguous formation of resin tags and poor micromechanical retention. [8,14,54] It is evident that the excess of proteins in HE act as a micromechanical (physical) and chemical barrier, leading to suboptimal bonding efficacy.[8,11,14,54]

The integration of a pretreatment protocol to remove intrinsic proteins has been advocated as a means to overcome these limitations.[15,31-33] Based on the positive outcomes associated with the use of 5% NaOCl in the management of hypocalcified amelogenesis imperfecta, few in vitro studies have investigated the oxidative effect of 5%–5.25% NaOCl on HE.[8,14,15,31] It is posited that the degradation of amino acids and hydrolysis of excess proteins cause a significant increase in the bond strength of HE, resulting in μSBSs that are comparable with those of NE.[8,14] This finding is not consistently observed in other laboratory studies; however, NaOCl pretreatment appears to decrease the risk of premature failures among HE specimens.[29]

Despite NaOCl being an effective deproteinising agent and its diverse applicability in dentistry, it is associated with various disadvantages.[14,48] Appropriate storage of NaOCl is often challenging, as it rapidly decomposes when exposed to air and sunlight, thus rendering it inactive.[48] It is also a potent oxidising agent, which may engender complications in the event of accidental spillage, including skin irritations or burns, damage to the oral mucosa, and injury to the eyes.[48] Extra precautions and preventive measures (eg, dental dam) are required to minimise the risk of harm, which may prove to be difficult especially in young children who have yet to develop cognitive-behavioural skills to cope with complex treatment procedures. The use of high-concentration NaOCl in hypomineralised young permanent molars with deep caries secondary to post-eruptive breakdown may also trigger an undesirable inflammatory effect on the large vital pulp.[48]

In contrast to NaOCl, Papacarie Duo gel is associated with superior advantages and has great relevance to paediatric dentistry. Randomised clinical trials investigating the effect and benefits of Papacarie Duo as a chemomechanical caries removal agent have proven it to be a cost-effective material that is capable of minimising treatment-associated pain in children.[2,4] Although no adverse effects have been reported, it should be used with prudence in patients with systemic conditions that may affect the host immune response, such as diabetes and blood disorders. Moreover, it is contraindicated in patients with G6PD (glucose-6-phosphate dehydrogenase) deficiency, as the presence of toluidine blue may incite an episode of haemolytic anaemia.

The favourable handling properties (ie, consistency and viscosity) of Papacarie Duo gel are postulated to play a pivotal role in allowing sufficient intra-/interprismatic and intratubular diffusion into enamel and dentinal tubules, respectively.[24,25] It has the capacity to decrease residual cariogenic bacterial load in dentin, while preserving the integrity of type I collagen and maintaining the mineral density of dentin, with no significant differences noted in Ca wt%, P wt% and Ca:P ratio.[24,25,46] It has also consistently given rise to promising experimental outcomes, including: 1. enhanced bonding efficacy of enamel and dentin; 2. excellent deproteinisation of dental substrate; 3. complete removal of the organic phase of smear layer, thus achieving adequate patency of dentinal tubules; and 4. a substantial improvement in the sealing performance of SE adhesives to enamel and dentin.[10,25,30,39]

In recent years, there has been a growing interest in the applicability and efficacy of Papacarie Duo gel as a deproteinising agent. Several in vitro studies have investigated its proteolytic effect on enamel specimens derived from extracted bovine incisors, human premolars, and MIH-affected FPMs.[3,14,42,43] These studies demonstrated a marked increase in the bond strength of pretreated enamel specimens; in particular, HE substrates were associated with more defined etching patterns and attained μSBSs that did not differ significantly from those of NE specimens.[14]

The μSBSs in the present study are congruous with these findings. NE and HE specimens exhibited a statistically significant increase in bond strength when the use of Papacarie Duo gel was followed by phosphoric acid etching in E&R mode. However, no significant effect was found on deproteinised enamel specimens when SU was applied in SE mode. Therefore, the second hypothesis, that deproteinising pretreatment of NE and HE would increase bond strength when conditioned on the same etching mode, was partially rejected.

Since the introduction of universal dental adhesives, there has also been a paradigm shift in the field of operative dentistry. These adhesives simultaneously promote the demineralisation of mineralized tissue, infiltration of resin monomers, and resin polymerisation.[26,38,45,47] As the matrix of universal dental adhesives is based on a combination of hydrophilic and hydrophobic monomers, they are able to concomitantly bond to both hydrophilic dental substrate and hydrophobic resin-based restorative material under various surface moisture conditions.[40,47,56]

To date, this study is the second in vitro experiment to determine the efficacy of a universal dental adhesive in association with MIH, and the first to investigate it with a deproteinising pretreatment protocol. Although results from

past studies using SU are primarily based on NE substrates derived from extracted bovine incisors and non-MIH-affected human teeth, their observations are in line with the current findings. Irrespective of the use of Papacarie Duo gel, both NE and HE specimens consistently showed significantly higher μSBS when SU was actively applied in E&R mode. The third hypothesis, that there would be a difference in bond strength between E&R mode and SE mode when conditioned on the same type of enamel substrate and subjected to the same pretreatment protocol, was supported.

Scrutinising the relevant μSBS data, the three hypotheses can be further extrapolated to CW HE and YB HE specimens for meaningful interpretation. In the absence of a deproteinising agent, the bond strength of CW HE was found to be significantly higher than that of YB HE. This is due to the disparity in the chemical composition of CW HE and YB HE substrates, with the more severe form of enamel hypomineralisation having a significantly higher protein content and markedly lower mineral density.[17,20,36]

The use of Papacarie Duo gel in conjunction with SE mode had a nonsignificant effect on both HE substrates. Contrarily, deproteinising pretreatment followed by phosphoric acid etching exerted the greatest effect on the bond strength of YB HE specimens, with μSBSs that were statistically comparable with those of CW HE specimens. This finding is congruent with the previous MIH in vitro study, which similarly examined the proteolytic effect of Papacarie Duo gel using a 2-step E&R dental adhesive.[14]

The overall mean μSBSs observed in this study are lower than previously published data.[8,14,54] The differences in results are attributable to the novel changes made to the microshear test methodology and related equipment. Even though these changes were undertaken to ensure that true shear force was delivered, the reliability of this modified methodology has yet to be determined. It would be valuable to conduct a finite element analysis and investigate the practicability of this modified methodology. Furthermore, it is difficult to compare current findings with other MIH in vitro studies, due to the large variability in the selection of restorative materials and dental adhesives, disinfecting solutions, storage conditions, specimen preparation techniques, and deproteinising pretreatment protocols.[23,49,50] These are some of the many confounding variables that can have an impact on μSBS.

Deproteinisation of dental enamel is still perceived as an uncommon practice in operative dentistry, and the timing of application (ie, before or after acid etching) remains unclear in literature.[8,31] SEM studies evaluating the deproteinising effect of NaOCl on various types of enamel substrates have reported increased surface roughness and better-defined etching patterns when NaOCl is used before phosphoric acid etching.[1,18,19] Extrapolating the understanding of Papacarie Duo gel to a hypothetical situation in which an MIH-affected tooth with post-eruptive breakdown and secondary caries is to be restored, it is postulated that the clinical workflow will first involve cavity preparation and atraumatic chemomechanical removal of caries using Papacarie Duo gel, followed by acid etching and then the remaining restorative procedure. Other non-MIH in vitro studies investigating the effect of deproteinisation on the shear bond strength of orthodontic brackets to normal human or bovine enamel have also supported the use of Papacarie Duo gel as a deproteinising agent prior to acid etching.[3,41-43] Hence, the pretreatment protocols of the present study were established in accordance with the experimental outcomes of previous studies.

Despite the variations and limitations within this study, the findings remain valid and relevant. The efficacy of Papacarie Duo gel as a deproteinising agent is well established, and in this study, it has shown great potential to improve the restorative outcomes of MIH-affected teeth. To optimise the bond strength of HE, it is recommended that Papacarie Duo gel be used as a pretreatment adjunct prior to the application of universal dental adhesives in E&R mode. Further MIH research investigating other universal dental adhesives, the timing of deproteinisation and newer enzymatic deproteinising agents, including papain-based Carie-Care (Uni-Biotech Pharmaceuticals; Chennai, India) and bromelain, are warranted.

Failure Mode

The majority of failures among NE specimens were either adhesive or mixed, with the latter being more common in groups subjected to deproteinising pretreatment and/or the application of SU in E&R mode. An increase in cohesive failure in resin composite was also found among deproteinised acid-etched NE specimens. These failure patterns indicate that NE is associated with higher enamel bond strength, and this is reflected by the μSBS results.

Conversely, 11 out of 80 HE specimens exhibited cohesive failure in enamel, of which 63.6% belonged to groups without deproteinising pretreatment. SEM evaluation of the debonded interface of these HE specimens showed fractured enamel rods, highlighting their compromised structure and associated low μSBS. Interestingly, the incorporation of a deproteinising agent led to a 2- to 2.5-fold increase in mixed failure mode and a 5% increase in cohesive failure within resin composite. These observations suggest that Papacarie Duo gel may help to increase the cohesive strength of bonded HE substrate. The failure pattern analysis of this study is reasonably congruent with those of past in vitro studies on MIH.[8,14,54]

CONCLUSION

The bond strength of resin composite to normal enamel and hypomineralised enamel was significantly increased when pretreatment with Papacarie Duo gel was followed by the application of Scotchbond Universal in etch-and-rinse mode. However, the bond strength of resin composite to hypomineralised enamel was consistently low when Scotchbond Universal was applied in self-etch mode, with or without deproteinising pretreatment.

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Clinical relevance: Papacarie Duo gel is capable of deproteinising HE. It should be considered in the restorative management of MIH as a pretreatment adjunct prior to phosphoric acid etching.