The influence of neutral MDP-Na salt on dentin bond performance and remineralization potential of etch-&-rinse adhesive

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Abstract

Objectives To investigate the effect of neutral 10-methacryloyloxydecyl dihydrogen phosphate salt (MDP-Na) on the dentin bond strength and remineralization potential of etch-&-rinse adhesive.

Methods Two experimental etch-&-rinse adhesives were formulated by incorporating 0 wt% (E0) or 20 wt% (E20) neutral MDP-Na into a basic primer. A commercial adhesive, Adper Single Bond 2 (SB, 3 M ESPE), served as the control. Sixty prepared teeth were randomly allocated into three groups ($n = 20$) and bonded using either one of the experimental adhesives or SB. Following 24 h of water storage, the bonded specimens were sectioned into resin-dentin sticks, with four resin-dentin sticks obtained from each tooth for microtensile bond strength (MTBS) test. Half of the sticks from each group were immediately subjected to tensile loading using a microtensile tester at a crosshead speed of 1 mm/min, while the other half underwent tensile loading after 6-month incubation in artificial saliva (AS). The degree of conversion (DC) of both the control and experimental adhesives ($n = 6$ in each group) and the adsorption properties of MDP-Na on the dentin organic matrix ($n = 5$ in each group) were determined using Fourier-transform infrared spectrometry. Furthermore, the effectiveness of neutral MDP-Na in promoting the mineralization of two-dimensional collagen fibrils and the adhesive-dentin interface was explored using transmission electron microscopy and selected-area electron diffraction. Two- and one-way ANOVA was employed to assess the impact of adhesive type and water storage on dentin bond strength and the DC ($\alpha = 0.05$).

Results The addition of MDP-Na into the primer increased both the short- and long-term MTBS of the experimental adhesives ($p = 0.00$). No difference was noted in the DC between the control, E0 and E20 groups ($p = 0.366$). The MDP-Na remained absorbed on the demineralized dentin even after thorough rinsing. The intra- and extra-fibrillar mineralization of the two-dimensional collagen fibril and dentin bond hybrid layer was confirmed by transmission electron microscopy and selected-area electron diffraction when the primer was added with MDP-Na.

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Conclusions The use of neutral MDP-Na results in high-quality hybrid layer that increase the dentin bond strength of etch-&-rinse adhesive and provides the adhesive with remineralizing capability. This approach may represent a suitable bonding strategy for improving the dentin bond strength and durability of etch-&-rinse adhesive.

Keywords Dentin bond strength, Etch-&-rinse adhesive, Remineralization, Degree of conversion

Introduction

Contemporary dental adhesives can be classified into two categories based on their bonding strategies to dental substrates: etch-&-rinse adhesive and self-etch adhesive [1]. Recently, manufacturers have introduced universal adhesives that can be used in both etch-&-rinse mode and self-etch mode [1]. Although self-etch approach is claimed to be user-friendly and less technique-sensitive, etch-&-rinse approach is the best choice for enamel bond and remains widely used in the clinic due to its proven clinical performance [2–4]. However, the dentin bond stability of etch-&-rinse approach is still suboptimal [5].

The etch-&-rinse adhesive use phosphoric acid etching to extract dentin minerals before the application of the adhesive. This procedure completely demineralizes the superficial dentin, exposes collagen fibrils, and activates the endogenous enzymes, such as matrix metalloproteinases (MMPs) and cysteine cathepsins (CC), in the dentin matrix [6, 7]. The monomer resin of the adhesive cannot completely infiltrate and encapsulate the demineralized collagen fibrils due to the considerable complexity of the supramolecular structures of the dentin matrix on the nanometric scale and the hydrophobic nature of the adhesive monomers [8–10]. The hybrid layer at the adhesive-dentin interface consists of exposed collagen fibrils, acid-activated dentin enzymes, and nano-channels full of loosely bonded water. The enzymatic degradation of the exposed collagen fibrils in the presence of water and the leaching of the resin from the hybrid layer leads to the degradation of the adhesive-dentin interface and eventually failure of the restorations [11].

Various experimental strategies have been proposed to enhance dentin bond durability, with biomimetic remineralization standing out as one of the most extensively studied approaches [12]. Biomimetic remineralization involves the use of biomimetic analogs (e.g., polyelectrolyte) that stabilize metastable amorphous mineral precursors to remineralize the inter- and intra-fibrillar portion of the collagen fibrils [13]. Researchers have resorted to solutions containing noncollagenous protein or template analogs to remineralize the exposed collagen fibrils at the adhesive-dentin interface, which is largely a proof of concept for this technology [14, 15]. To facilitate the transition of this proof-of-concept into a clinically applicable approach, researchers have synthesized experimental adhesives added with bioactive components, such as calcium phosphate [16] and phosphoprotein biomimetic analogs, to preserve the hybrid layer longevity in

the follow-up studies [17, 18]. However, the additional bioactive components have a weakening effect on resins and compromise the immediate bond strength of adhesive due to the limited chemical interaction between the biofunctional components and resin [17, 18]. A preceding meta-analysis revealed that, while the mineralization strategy mitigates dentin bond strength loss after aging, it concurrently diminishes the immediate dentin bond strength [19]. Therefore, it is certainly worth looking for new biomimetic analogs which could endow the dental adhesive with remineralizing ability without compromising the initial dentin bond strength.

Ten-methacryloyloxydecyl dihydrogen phosphate (MDP) is one of the most excellent acidic functional monomers which could demineralize the tooth surface and simultaneously form a strong chemical bond to the hydroxyapatite by stable MDP-Ca salts [20]. The MDP monomer is mostly used in self-etch adhesives and universal adhesives [21, 22]. The chemical bonding of MDP and its neutral MDP-Na salts with hydroxyapatite surfaces has been demonstrated before; however, the effect of chemical bonding of neutral MDP-Na on dentin matrix and dentin bond strengths has not been elucidated yet [23]. Most recently, our previous study demonstrated that MDP monomers have dual functions, that is, de- and re-mineralizing abilities [24]. This hints that either a MDP monomer or its polymer (poly-MDP) as a polyelectrolyte could induce mineralization [24].

Herein, we aimed to evaluate the effects of neutral MDP-Na salt on the dentin bond strength and remineralization potential of experimental etch-&-rinse adhesive. The null hypothesis to be tested was that (1) the neutral MDP-Na salt did not affect the short- and long-term dentin bond strength of the experimental etch-&-rinse adhesive and (2) the neutral MDP-Na salt could not endow the experimental etch-&-rinse adhesive with biomimetic remineralization potential at the adhesive-dentin interface.

Materials and methods

The study was conducted in accordance with the International Ethical Guidelines and Declaration of Helsinki and approved by the Institutional Ethics Committee. The experimental flowchart is presented in Fig. 1.

Tooth preparation

In total, 75 caries-free, crack-free, freshly extracted human third molars were collected with patients'
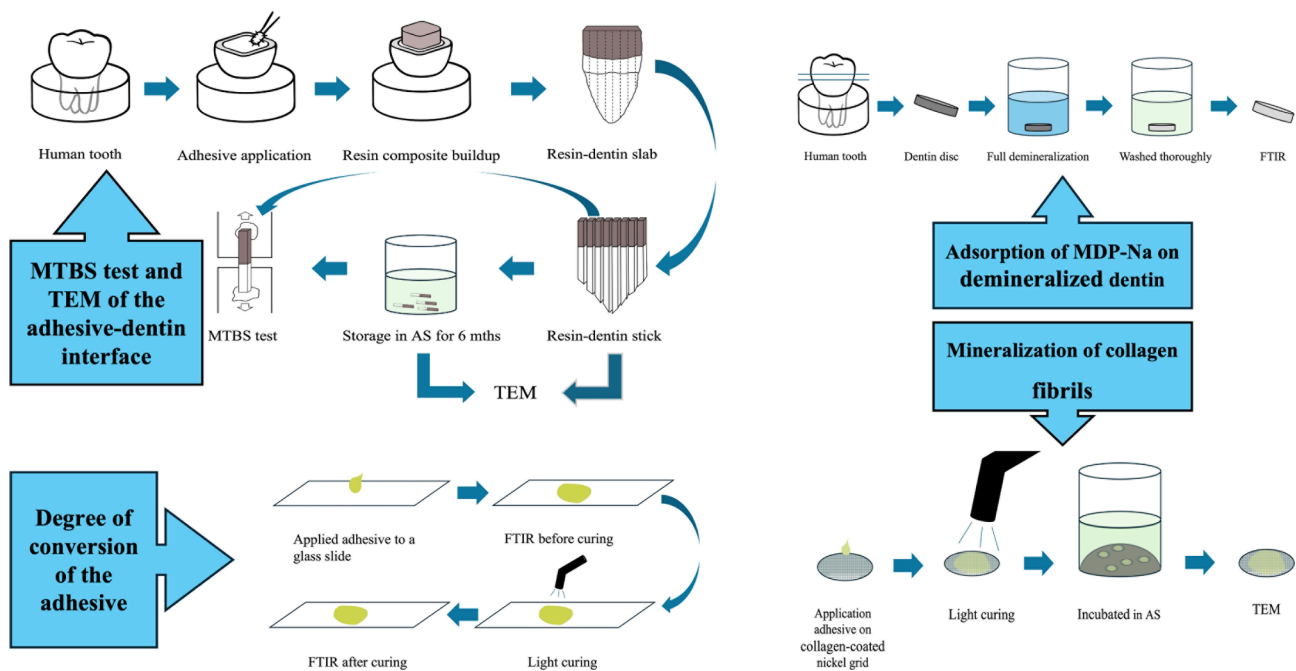
Fig. 1 - The experimental flowchart

informed consent. The teeth were stored in 0.5% chloramine T solution at 4 °C and used within 1 mth after extraction. The mid-coronal dentin was exposed using a slow speed diamond saw (Isomet 1000, Buehler Ltd., Lake Bluff, IL, USA) and polished with 600-grit SiC paper under running water for 30 s. To ensure that the area used for bonding was free of any remaining enamel, an inspection was conducted on the exposed dentin surface using a stereo microscope (Leica MZ APO, Germany) at 50-fold magnifications.

Preparation of experimental adhesives
A 25 wt% of the MDP solution was prepared by dissolving 5 g MDP (Watson International Ltd, Jiangsu, China) in 15 g 50 wt% ethanol. The pH value of the solution was adjusted to 7.0 using 1 M sodium hydroxide; then, the solution was lyophilized to obtain a neutral MDP-Na salt.

Two three-step experimental adhesives comprising primers and adhesive resin were prepared. The adhesive resin included Bisphenol A glycerolate dimethacrylate (Bis-GMA; MilliporeSigma, St. Louis, MO, USA) and 2-hydroxyethyl methacrylate (HEMA; MilliporeSigma, St. Louis, MO, USA) with a mass ratio of 55/45 and was formulated with 0.5 wt% camphorquinone (CQ; MilliporeSigma, St. Louis, MO, USA) and 0.5 wt% ethyl-4-dimethylaminobenzoate (EDMAB; MilliporeSigma, St. Louis, MO, USA) as photoinitiators. A basic experimental primer (E0) without MDP-Na salt was created by blending 10 g of experimental adhesive resin with 5 g of ethanol and 5 g of distilled water. Subsequently, an

additional experimental primer (E20) was formulated by blending 2 g of MDP-Na salt with 8 g of the basic primer. The final concentration of each component in the experimental primer was 21.78 wt% GMA, 17.82 wt% HEMA, 0.2 wt% CQ, 0.2 wt% EDMAB, 20 wt% water, 20 wt% ethanol, 20 wt% MDP-Na.

Microtensile bond strength testing
Sixty prepared teeth were randomly divided into 3 groups ($n=20$): one control group (Adper Single Bond 2 (SB), 3 M ESPE) and two experimental groups. The dentin surface was etched using 37% phosphoric acid (Aladdin, Shanghai, China) for 15 s. Afterward, it was rinsed with water for 30 s and any excess water was removed by blotting with a cotton pellet on one side of the specimen. In the control group, two consecutive coats of the adhesive were applied to the dentin surface for 15 s with gentle agitation, followed by gentle air-thinning for 5 s to evaporate the solvent and light curing for 10 s using a light-curing unit (Bisco Inc., Schaumburg, IL, USA) with a light intensity of approximately 600 mW/cm². For the experimental groups, the conditioned dentin surface was applied with one of the above-mentioned primers, agitated for 15 s, and gently air-dried for 5 s before it was applied with the adhesive resin, gently air-thinned, and light cured for 10 s. Afterward, a resin composite (Filtek Z250, 3 M Oral Care) was placed over the adhesive-coated dentin surface in four 1-mm thick increments; each increment was polymerized for 20 s. After 24 h of storage in distilled water at 37 °C, all the dentin-bonded

specimens were vertically sectioned into 0.9-mm thick resin-dentin slabs, and two slabs adjacent to the center of each tooth were further sectioned into multiple sticks with a cross-sectional area of approximately 0.9×0.9 mm. The four longest resin-dentin sticks from those two slabs were used to represent the microtensile bond strength of that particular tooth. Half of the resin-dentin sticks were subjected to tensile loading with a microtensile tester (Bisco, Schaumburg, IL, USA) at a crosshead speed of 1 mm/min until failure. The remaining sticks were tested under tensile loading after storage in weekly changed AS solution (1.5 mM $CaCl_2$, 0.9 mM KH_2PO_4, 20 mM HEPES, 260 mM KCl, and 2 mM NaN_3; pH=7.0) at 37 ℃ for 6 mths. No pre-testing failure occurred in the present study. The Microtensile bond strength (MTBS) was calculated in megapascals (MPa).

Degree of conversion (DC)

The DC of the control and experimental adhesives were determined using attenuated total reflectance–Fourier transform infrared (ATR-FTIR) spectroscopy. The control (SB), E0 and E20 primers were each smeared on a glass slide and air-dried for 5 s, respectively; subsequently, the experimental adhesive resin was applied onto the primer-treated surface and gently air-thinned. Before curing, the treated surfaces were determined by ATR-FTIR with a wavelength of 500–4000 cm^{-1}, a resolution of 4 cm^{-1}, and 32 scans to acquire the reference (1608 cm^{-1}, aromatic C=C) and reaction (1638 cm^{-1}, aliphatic C=C) peaks of the mixture of the experimental primer and adhesive resin. Subsequently, the specimens were cured with a light curing unit (Bisco Inc., Schaumburg, IL, USA) for 10 s and stored in a light-proof container (*n*=6) for 24 h before they were determined by ATR-FTIR. DC was calculated using the following formula: DC (%) = [1 − (Area of band C=C/area of band C=O) polymer/(Area of band C=C/area of band C=O) monomer] × 100.

Adsorption characteristics of MDP-Na on demineralized dentin

Two additional experimental primers were prepared by blending MDP-Na or HEMA with 50 wt% ethanol in the ratio of 20 wt%. For this, 15 human third molars were cut parallel to the occlusal surfaces between the occlusal and middle third and sectioned into dentin discs (1-mm thick) with a slow speed diamond saw (Isomet 1000, Buehler Ltd., Lake Bluff, IL, USA). One disc was acquired from each tooth. After the dentin surface was polished with 600-grit silicon carbide paper, the discs were immersed in a 10% phosphoric acid (Aladdin, Shanghai, China) solution for 24 h to completely demineralize the dentin. The full demineralization of the dentin discs was validated by the absence of any white precipitate

formation upon the incremental addition of a 10% potassium oxalate solution to the demineralization solution [25, 26]. After rinsing with distilled water, the specimens were randomly divided into three groups (*n*=5): Specimens were immersed in tubes filled with 10 mL distilled water, 10 mL 20 wt% MDP-Na, or 10 mL 20 wt% HEMA solution. All the specimens in the tubes were rotated end over end for 24 h at 37 ℃, then rinsed with distilled water for 30 s to remove any residual debris, and ultrasonically cleansed in 50 mL of 50% ethanol-aqueous solution for 24 h, with the solution being changed every 8 h. The washed specimens were completely dried in an oven at 37 ℃ for 48 h. Finally, the dried specimens were analyzed using ATR-FTIR.

Mineralization of collagen fibrils

The control adhesive, E0 and E20 primers were used for the mineralization of collagen fibrils. A single-layer reconstructed collagen model was prepared according to our laboratory protocol [24, 27]. Briefly, rat tail type I collagen solution (3 mg/mL, Gibco, Invitrogen) was diluted to 50 μg/mL in 0.5 mL of assembling solution (pH 9.2, 50 mM glycine, 200 mM KCl), kept at room temperature for 20 min, and then 3 μL droplets of the collagen solution were added to 400-mesh nickel grids coated with formvar/carbon film (Beijing Zhongjingkeyi Technology Co., Ltd., China). The grids were left in a petri dish overnight in a 100% humidity chamber at 37 ℃. Collagen crosslinking was done with 0.05 wt% glutaraldehyde for 1 h. The collagen-coated grids were then thoroughly washed with distilled water and air-dried. Self-assembled collagen fibrils were confirmed by TEM (JEM-1230, JEOL, Tokyo, Japan) after randomly selecting several collagen-coated grids and staining them with 1% uranyl acetate for 15 s. The collagen-coated transmission electron microscopy (TEM) grids were pretreated with the control adhesive or one of the mixtures of the experimental primer and adhesive resin in a volume ratio of 1:1 for 10 min, gently air-thinned for 5 s, and light-cured for 10 s. Afterward, the TEM grids were each incubated in 120 μl of daily-changed AS at 37 ℃ for 7, 14, and 28 d. Three TEM grids from each group were retrieved at a predetermined time and washed in sequence with distilled water, 50% and 100% ethanol for 5 s each. All specimens were examined using TEM (JEM-1230, JEOL, Tokyo, Japan) at 110 kV. Selected area electron diffraction (SAED) patterns were recorded using high-resolution TEM (JEM-2100 F, JEOL, Tokyo, Japan).

TEM of the adhesive-dentin interface

Three resin-dentin sticks from each bonding group were fixed in Karnovsky's fixative both before and after a 6-month storage in AS. Subsequently, the specimens were washed thrice in sodium cacodylate buffer before

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Table 1 The means and standard deviations (means ± SD) of dentin MTBSs of the control and experimental groups

Adhesive system	Storage time	
	24 h	6 mths
Control (SB)	57.07 ± 4.58[Aa]	48.71 ± 6.67[Aa]
0 wt% MDP-Na (E0)	51.89 ± 6.77[Aa]	32.1 ± 7.93[Bb]
20 wt% MDP-Na (E20)	85.25 ± 4.95[Ba]	61.41 ± 6.67[Cb]

Different uppercase letters in columns denote significant differences among various bonding groups ($p < 0.05$). Different lowercase letters within rows signify significant differences in storage times within each bonding group ($p < 0.05$)
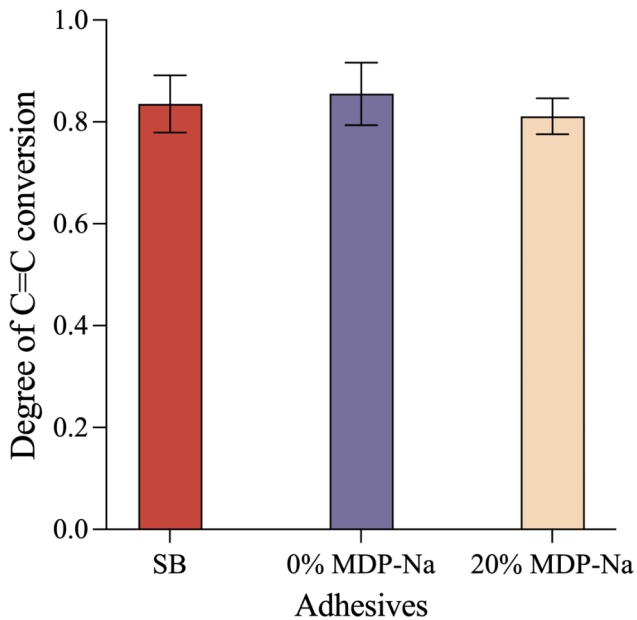
Fig. 2 - The DC of the control and experimental groups after light curing

they were dehydrated in ascending grades of ethanol (50–100%), immersed in propylene oxide as a transitional fluid, and then embedded in epoxy resin. Non-demineralized sections with a thickness of 70–90 nm were prepared and examined without further staining using TEM.

Statistical analysis
Statistical analysis was performed using SPSS version 24 (SPSS Inc., IL, USA). The four sticks from a single tooth were treated as one unit for analysis. Data sets from each experiment were initially assessed for normality and homoscedasticity before applying parametric statistical methods. If either assumption was violated, the data sets were non-linearly transformed to meet these criteria prior to further analysis. Two-way ANOVA was employed to assess the impact of adhesive type and AS incubation on dentin bond strength. Additionally, one-way ANOVA was utilized to investigate the influence of MDP-Na on both short- and long-term dentin bond strength, as well as on the DC, followed by Tukey's post

hoc test for conducting multiple comparisons. Statistical significance was preset at $\alpha = 0.05$.

Results
MTBS
The addition of neutral MDP-Na salt to the basic primer significantly increased the immediate dentin MTBS from 51.89 ± 7.78 to 83.57 ± 6.67 MPa ($p = 0.00$) (Table 1). Following a 6-month storage in AS, there was a notable decline in the dentin bond strength of the control group ($p = 0.006$) and the experimental groups ($p = 0.00$). Particularly, E0 group exhibited the most significant deterioration, while the MTBS of the E20 group remained higher than that of the E0 group ($p = 0.00$).

DC
The DC of the adhesives are shown in Fig. 2. The ATR-FTIR spectra of the control, E0 and E20 groups showed a mean DC of $83.5\% \pm 7.9\%$, $85.5\% \pm 5.6\%$ and $81.1\% \pm 3.2\%$ respectively. No statistically significant differences in DC were found between the three groups ($p = 0.366$).

Adsorption characteristics of MDP-Na on demineralized dentin
Figure 3 shows the ATR-FTIR spectra of the demineralized dentin treated with the resin monomers. The C=O stretching vibration (at 1716 cm^{-1}) associated with the methacryloxy carbonyl group was observed in the neat MDP-Na and HEMA but absent on the untreated dentin surface. Following the reaction of the respective resin monomer with the demineralized dentin, accompanied by thorough rinsing with ethanol aqueous solution, the methacryloxy carbonyl peak persisted in the MDP-Na-treated dentin while it diminished in the HEMA-treated dentin.

Mineralization of collagen fibrils
After the reconstituted type I collagen fibrils were pretreated with the experimental adhesive containing MDP-Na, polymerized, and further incubated in AS for 7 d, the collagen fibrils were partially mineralized with a characteristic banding pattern between the mineralized and unmineralized parts (Fig. 4A). After further incubation for 14 d, the collagen fibrils were heavily mineralized but were in a noncrystalline state (Fig. 4B). After further incubation in the AS solution for 28 d, most of the collagen fibrils were mineralized with electron-dense needle-like minerals aligned in parallel along the c-axis of the collagen fibrils (Fig. 4C, Fig. S2A). The SAED pattern of the mineralized collagen fibrils (Fig. 4C) produced an arc-shaped diffraction pattern of the (002) growth face. In contrast, mineralization of collagen fibrils was not observed in the control and E0 group, even after 28 d (Fig. S1).
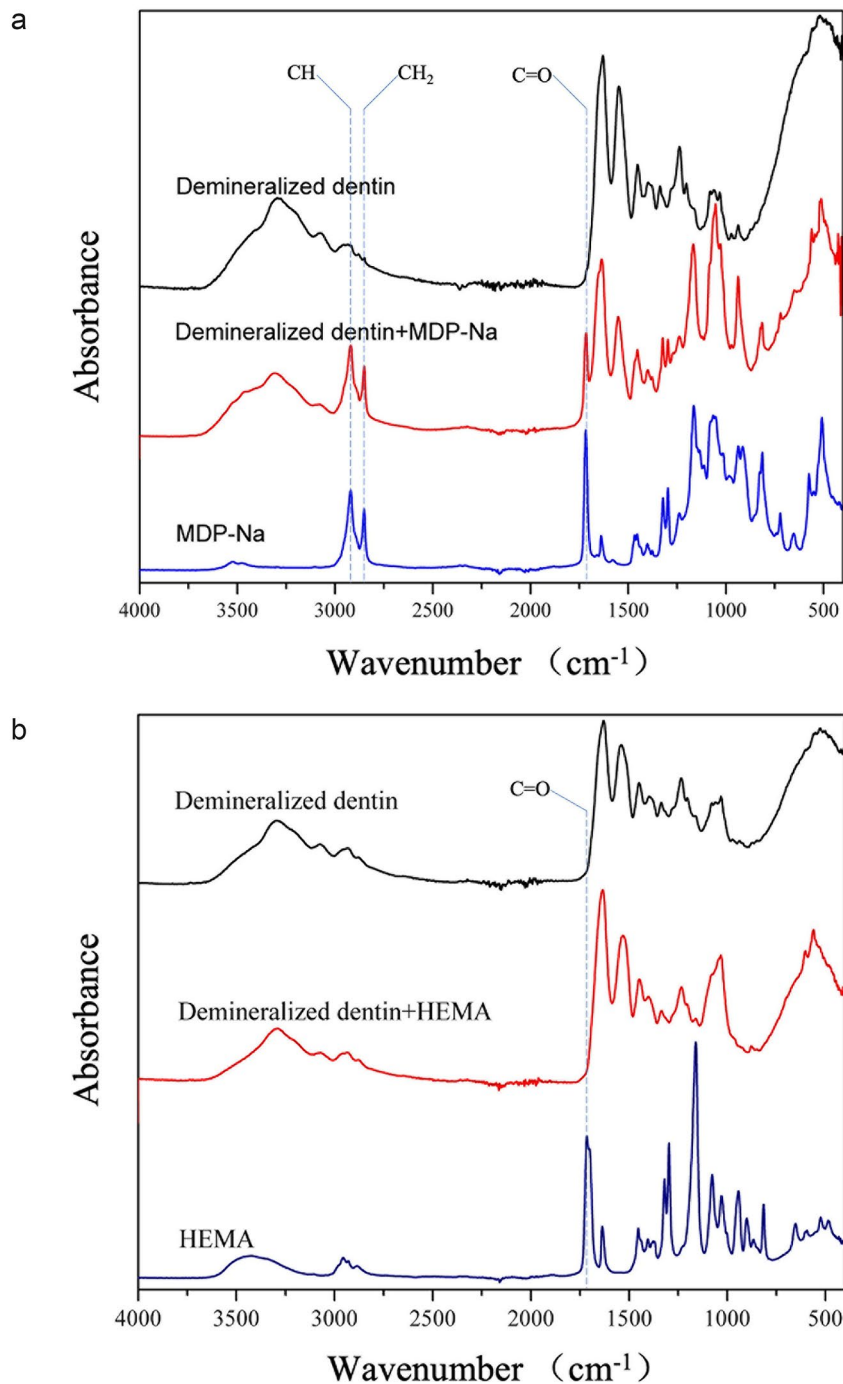
Fig. 3 - The ATR-FTIR spectra of demineralized dentin, MDP-Na, MDP-Na-treated demineralized dentin, HEMA, and HEMA-treated demineralized dentin

Remineralization of the adhesive-dentin interface

The TEM images reveal an electron-lucent hybrid layer in the control and experimental groups before remineralization (Fig. S3A, B, C). After 6-month incubation, the hybrid layer of the control and E0 group remained electron-lucent in general (Fig. 5A, B). Conversely, newly formed crystals could be detected in the hybrid layer of the E20 group (Fig. 5C).

Discussion

Regarding the etch-&-rinse adhesive, two bonding mechanisms are responsible for the bond strength: main mechanical interlocking and additional chemical bonding [28]. In this study, the dentin MTBS results demonstrated that the primers fortified with neutral MDP-Na salt exhibited notably higher dentin bond strengths both after 24 h water-storage and following a 6-month
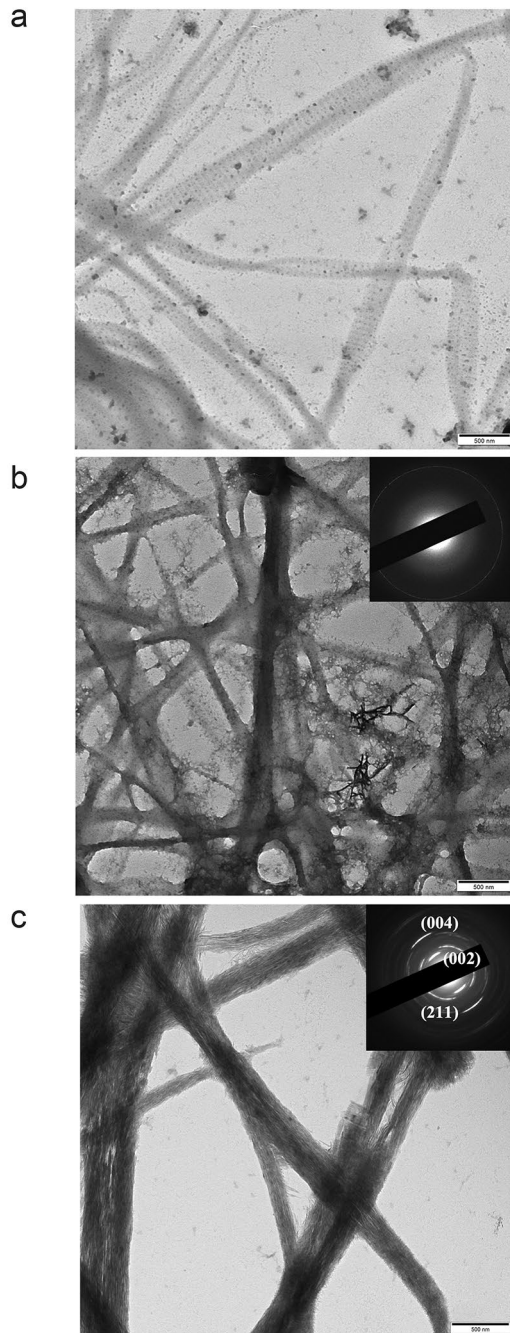
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Fig. 4 - The TEM images of the remineralization of the reconstituted type I collagen fibrils using the MDP-Na-containing adhesive. The collagen fibrils became mineralized with a characteristic banding pattern after 7 days of incubation (**A**). The amounts of mineralization of collagen fibrils increased after 14 days; the banding pattern of the collagen fibrils was not detected, and the minerals were in an amorphous form (**B**). The collagen fibrils were heavily mineralized after 28 days, and the crystals aligned along the longitudinal axis of the collagen fibrils. The selected-area electron diffraction of these crystals produced ring patterns consistent with the crystalline apatite's characteristic features (**C**)
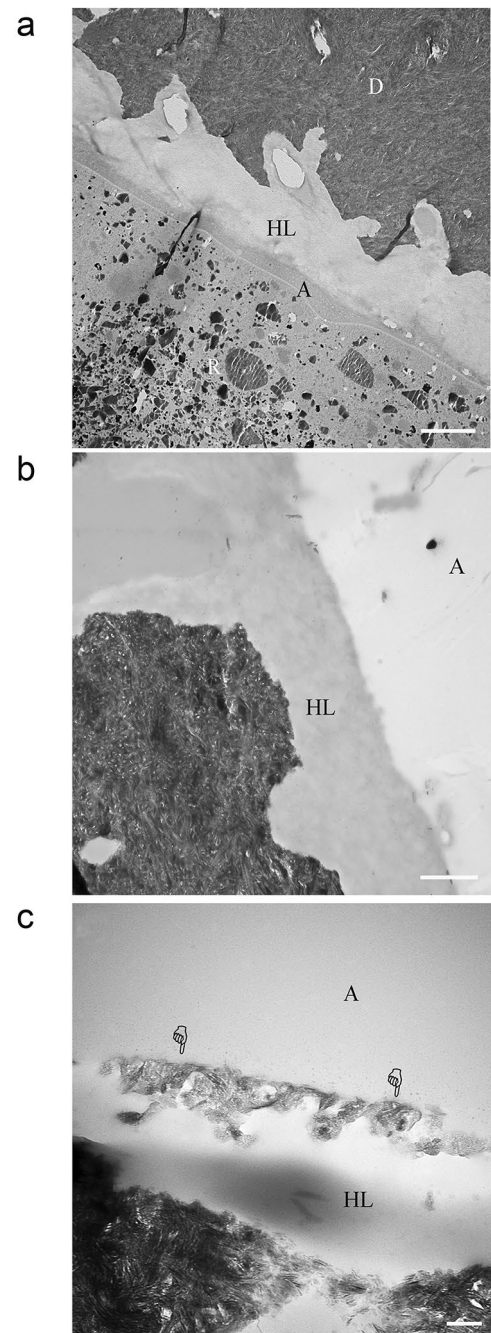

Fig. 5 - TEM images of the resin-dentin interfaces after 6-month incubation in the AS. No evidence of remineralization was observed in the hybrid layer of the control (**A**, bar = 5 μm) and 0 wt% MDP-Na (**B**, bar = 5 μm) group while non-uniform remineralization was apparent in the hybrid layer of the 20 wt% MDP-Na group, with distinct regions exhibiting substantial remineralization (pointers) (**C**, bar = 2 μm). A: adhesive layer, HL: hybrid layer, D: intertubular dentin

aging period compared to the primer without MDP-Na (Table 1). Thus, the null hypothesis that the neutral MDP-Na salt did not affect the short- and long-term dentin bond strength of the experimental etch-&-rinse adhesive was rejected. This notable enhancement likely

arises from the chemical bonding facilitated by MDP-Na salt with hydroxyapatite, aligning with previous reports [23, 29]. The significance of chemical bonding between MDP monomers and hydroxyapatite (HA) surfaces in augmenting dental bonding effectiveness has been well-documented [30, 31]. Studies, such as Wang et al.'s investigation, have confirmed the ability of neutral MDP-Na to achieve bond strength solely through chemical adsorption onto enamel hydroxyapatite [29]. In the present study, phosphoric acid etching completely demineralized the dentin surface; however, there were still lots of hydroxyapatite crystals at the bottom of the hybrid layer. This will provide potential chemical bonding sites for MDP-Na [23, 32]. Besides, the addition of 20% of MDP-Na did not affect the DC of the adhesives (Fig. 2), which was positively correlated with the mechanical strength of the adhesive layer and bond strength.

Furthermore, chemical bonding between functional monomers and dentin organics has also recently attracted much attention [27, 33]. Stable chemical interaction between these functional monomers and the exposed collagen matrix could produce higher dentin bond strength [33, 34]. The ATR-FTIR spectra in this study indicated stable adsorption of neutral MDP salt onto the dentin organic matrix (Fig. 3). The adsorption mechanism of MDP molecules on dentin collagen involves several key interactions. Firstly, MDP contains phosphate groups capable of forming hydrogen bonds with the amino acid residues in the collagen fibrils of dentin, anchoring the adhesive to the organic matrix. Secondly, the methacrylate group of MDP interacts with hydrophobic regions of collagen, facilitated by the long alkyl chain of MDP, which enhances the penetration and stability of the adhesive within the collagen matrix. Additionally, there is the potential for new covalent bonds (P−N bonds) to form between the phosphate groups of the monomers and the NH_2 groups in collagen [35−37].

Although acid-etching is necessary for dentin bonding, it opens a Pandora's box of collagen fibrils, matrix metalloproteinases, cysteine cathepsins, and water [38]. The dentin bonding interface rapidly degrades over time due to the exposure of dentin collagen fibrils and activation of endogenous proteases [38]. Collagen fibrils stabilized by intrafibrillar and interfibrillar apatite crystallites in mineralized tissues do not degrade over time [39]; thus, the biomimetic remineralization strategy has been used to improve the dentin bond durability [40−42].

Up to now, there have been two major mineralization strategies to improve the dentin bond durability: pretreating solutions and incorporating mineral-promoting contents into adhesive [14, 15]. While the former is not suitable for clinical practice, the latter may jeopardize the immediate bond strength of the adhesive [16−18]. In the present study, the MDP-Na salt contained in the primer probably has co-polymerized with other resin monomers, avoiding the adverse effect of additional components on the adhesive-dentin interface [43]. Thus, the E20 primer did not decrease the immediate dentin MTBS and significantly increased the bond strength (Table 1). Furthermore, the morphology and orientation of the hydroxyapatite crystals confirmed the inter-and intra-fibrillar mineralization of the reconstituted type I collagen fibrils (Fig. 4, Fig. S2) [44]. For the adhesive-dentin interface, both the control and experimental groups presented an electron-lucent hybrid layer before remineralization (Fig. S3). After 6 mths of immersion in the AS solution, remineralization could be identified in the hybrid layer of the E20 group, which indicated that the Ca/P ion within the AS solution can diffuse into the water-filled regions of the hybrid layers to remineralize the exposed collagen fibrils (Fig. 5). This remineralization process has the potential to deactivate or stabilize the endogenous MMP and cysteine within the hybrid layer, contributing to the enhanced stability of the adhesive-dentin interface [40, 45, 46].

In the hybrid layer, MDP exists as either a monomer or a polymer. The former is capable of permeating dentin collagen fibrils, disrupting the triple helical structure by cleaving hydrogen bonds, and ultimately becoming immobilized within the collagen matrix [24]. The MDP-bond collagen acts as a substantial collagenous phosphoprotein, effectively capturing Ca^{2+} ions and phosphate groups or amorphous Ca/P precursors through electrostatic attraction. This process can induce biomimetic mineralization of collagen fibrils without the need for non-collagenous proteins or polymer additives [24]. Additionally, the poly-MDP may serve as a carrier for amorphous calcium phosphate (ACP) nanoprecursors, facilitating the delivery of biomimetic remineralization to demineralized dentin [27, 47]. Anyhow, the findings in this study demonstrated that the MDP-Na and its polymer could induce the mineralization of the reconstituted collagen fibrils and the hybrid layer at the adhesive-dentin interface (Fig. 5), which is consistent with previous studies [24, 27, 47]. Thus, the null hypothesis that the neutral MDP-Na salt cannot endow the etch-&-rinse adhesive biomimetic remineralization potential was rejected.

Recently, manufacturers have incorporated increasing concentrations of hydrophilic monomers to make these adhesives more compatible for bonding to intrinsically moist, acid-etched dentin. However, with the increase of the hydrophilic nature of the adhesive, a weak linkage is created at the adhesive-dentin interface because the hydrophilic resin monomers are vulnerable to hydrolysis [1]. An "ideal" dental adhesive should be a hydrophilic monomer that can interact with moist dentin surfaces and become completely hydrophobic once it is

polymerized to discourage water sorption and hydrolysis. To some extent, MDP-Na has this property as the soluble MDP-Na absorbs calcium ions in simulated body fluid and forms insoluble MDP-Ca salts. Moreover, unlike the self-etch adhesive, there is no soluble calcium hydrogen phosphate formed in this process [48, 49], which may affect the stability of the hybrid layer [50].

The present study had some limitations. In vitro models cannot fully simulate the in vivo conditions. From a clinical perspective, further research is necessary to investigate the effect of neutral MDP-Na on the dentin bonding performance of two-step etch-&-rinse adhesive, as well as its impact on enamel bonding. Additionally, the absence of failure mode analysis makes it difficult to identify the potential weaknesses in the adhesive-dentin interface, so future studies should place greater emphasis on fractography analysis and bond interface examination.

Conclusion

The MDP-Na containing primer could not only greatly improve the short- and long-term dentin bond strength of the experimental etch-&-rinse adhesive but also imbues the adhesive with the potential for remineralization at the adhesive-dentin interface.

Supplementary Information

The online version contains supplementary material available at https://doi.org/10.1186/s12903-024-04756-y.

Supplementary Material 1

Declarations

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