Journal of Dentistry 60 (2017) 77–86

Contents lists available at ScienceDirect

Journal of Dentistry

journal homepage: www.intl.elsevierhealth.com/journals/jden

Effect of a novel quaternary ammonium silane cavity disinfectant on durability of resin–dentine bond

D. Daood[a], C.K.Y. Yiu[a,*], M.F. Burrow[b], L.-N. Niu[c], F.R. Tay[d]

[a] Paediatric Dentistry and Orthodontics, Faculty of Dentistry, The University of Hong Kong, Prince Philip Dental Hospital, 34 Hospital Road, Pokfulam, Hong Kong Special Administrative Region
[b] Melbourne Dental School, The University of Melbourne, Australia
[c] State Key Laboratory of Military Stomatology, Department of Prosthodontics, Fourth Military Medical University, Xi'an, Shaanxi, PR China
[d] Department of Endodontics, The Dental College of Georgia, Augusta University, Augusta, GA, USA

ARTICLE INFO

Article history:
Received 23 December 2016
Received in revised form 26 February 2017
Accepted 5 March 2017

Keywords:
Cysteine cathepsins
Dentine
Quaternary ammonium silane
Matrix metalloproteinases

ABSTRACT

Objective: The present study examined the effect of a quaternary ammonium silane (QAS) cavity disinfectant on the viability of human dental pulp cells, dentine bond durability and nanoleakage of simplified etch-and-rinse adhesives.
Methods: Etched dentine surface of third molars were randomly divided into two adhesive groups, Adper™ Single Bond 2 and Prime & Bond® NT™. For each adhesive, the teeth were randomly assigned to five cavity disinfectant groups ($N = 6$): Group 1: deionised water (control); Group 2: 2% chlorhexidine (CHX); Group 3: 2% QAS; Group 4: 5% QAS and Group 5: 10% QAS. The cavity disinfectants were applied on etched dentine surfaces for 20 s, followed by adhesive application. The bonded teeth were sectioned for bond strength testing at 24 h, 6 months and 12 months. Viability of human dental pulpal cells was examined using MTT assay. Bond strength data were analysed using 3-way ANOVA and Tukey test. Interfacial nanoleakage was evaluated after 24 h and 12 months and analysed using Kruskal–Wallis test.
Results: Significant differences in bond strength were observed for the factors disinfectants ($p < 0.001$) and time ($p < 0.001$); while the factor, adhesive, was not significantly different ($p = 0.203$). The 2% QAS cavity disinfectant preserved bond strength of both adhesives and reduced interfacial nanoleakage after 12 months. Cell viability was the lowest for 2% CHX, followed by 2% QAS and the control.
Conclusions: The 2% QAS cavity disinfectant demonstrated greater cell viability compared to 2% CHX, with no adverse effect on immediate bond strength and preserved bond stability over time.
Clinical significance: Incorporation of 2% quaternary ammonium silane cavity disinfectant in the resin–dentine bonding protocol enhances the success rate of bonded restorations.

1. Introduction

The integrity of the adhesive bond between the tooth substrate and resin is pivotal to the durability of resin-based restorations. Despite the popularity of tooth-coloured restorations, secondary caries at tooth-restoration margins is still the primary reason for replacement of resin composite restorations [1]. Incomplete removal of caries-infected dentine during cavity preparation may result in entrapment of bacteria within the cavity. Residual bacterial colonies within the smear layer and dentinal tubules produce degenerative products, such as lipoteicholic acids and endotoxins that diffuse into the dental pulp, causing irritation and inflammation [2]. Proliferation of remnant bacteria may result in leakage at restorative margins, causing secondary caries and restoration failure over time [3,4]. Hence, disinfection of a cavity preparation with an antimicrobial agent has been recommended prior to the restorative procedures [5].

Chlorhexidine (CHX) is commonly used as a cavity disinfectant to eliminate residual bacteria in caries-affected dentine following mechanical caries removal [6]. Chlorhexidine is a potent and broad spectrum antimicrobial agent against oral bacteria, notably *Streptococcus mutans* [7]. Being an inhibitor of dentine matrix-bound matrix metalloproteinases [8,9] and cysteine cathepsins [10,11], CHX plays an important role in preserving the integrity of resin–dentine bond over time. The substantivity of CHX is due to its ability to bind to tissues surfaces [12]. However, CHX only binds electrostatically to dentine collagen and may be displaced by

* Corresponding author at: Paediatric Dentistry and Orthodontics, Faculty of Dentistry, The University of Hong Kong, Prince Philip Dental Hospital, 34 Hospital Road, Pokfulam, Hong Kong Special Administrative Region.
E-mail address: ckyyiu@hkucc.hku.hk (C.K.Y. Yiu).

http://dx.doi.org/10.1016/j.jdent.2017.03.003
0300-5712/

competing cations from dentinal fluid and saliva [13], thereby compromising its antimicrobial and protease inhibitory effects [14]. Furthermore, CHX has been shown to exert dose-dependent, mild transdentinal toxic effects on odontoblast-like cells [15]. Hence, there is a need to look for alternative antibacterial cavity cleansers to inhibit oral biofilms and caries.

The quaternary ammonium compound, 3-(trimethoxysilyl)-propyldimethyloctadecyl ammonium chloride (SiQAC; $C_{26}H_{58}ClNO_3Si$; CAS Registry Number 27668-52-6), is commonly used as antimicrobial coatings for medical devices [16–18] and garment fabrics [18,19] because of its low in vivo toxicity profile [20]. Being a trialkoxysilane, SiQAC possesses hydrolysable and condensable methoxy groups, which enable it to covalently attach to other alkoxysilanes or silanol-containing substrate surfaces via the formation of siloxane bridges [21]. The antimicrobial property of SiQAC may be attributed to its long, lipophilic C18 alkyl chain. The latter penetrates bacterial cell membrane and causes cell death via a contact-killing mechanism [22]. Recently, SiQAC has been coupled to other trialkoxysilanes with methacryloxy or epoxy functionalities via sol–gel platform chemistry that utilises tetraethoxysilane (TEOS) or dimethyldiethoxysilane as the anchoring unit [23–28]. Such sol–gel reactions between one tetraalkoxy silane and two trialkoxysilanes generated a host of antimicrobial quaternary ammonium silane (QAS) molecules with methyacryloxy or epoxy functionalities that can copolymerise with methacrylate or epoxy resins.

Using similar platform chemistry, an ethanol- or acetone-soluble, fully-hydrolysed, partially-condensed version of QAS has been synthesised by the authors. The synthesis involves reaction of TEOS with 3-(triethoxysilyl)-propyldimethyloctadecyl ammonium chloride (i.e. the ethoxy version of SiQAC, abbreviated as Et-SiQAC). Because the hydrolysis by-product of this sol–gel reaction is ethanol instead of methanol, the QAS (codenamed K21; CAS Registry Number 1566577-36-3; IUPAC name: 1-octadecanaminium, N,N′-[[3,3-bis [[[3-(dimethyloctadecylammonio) propyl] dihydroxysilyl] oxy]-1,1,5,5,-tetrahydroxy-1,5-trisiloxanediyl] di-3,1-propanediyl] bis [N,N-dimethyl] chloride (1:4); Fig. 1A) may be used, without further purification, as an intra-oral cavity disinfectant. Unlike the SiQAC or Et-SiQAC molecule, inclusion of TEOS as the network-forming agent enables a three-dimensional organically modified silicate to be produced by condensation of additional tetra- and triethoxysilane molecules with remnant silanol groups within the molecule.

When the QAS cavity disinfectant is applied to smear layer-depleted dentine, progressive condensation of the 3-D organically-modified silicate network may result in occlusion of hybrid layers and dentinal tubules that are not completely infiltrated by dentine bonding agents, with the potential of reducing nanoleakage and dentine hypersensitivity. Based on the contact-killing antimicrobial activities exhibited by SiQAC-derived macromolecules [24–29], it is anticipated that the QAS cavity disinfectant may be used on caries-infected dentine [29,30], caries-affected dentine, as well as sound dentine, where ingress of bacteria may occur via leaking cavosurface margins. Being a molecule without methacryloxy functional groups, the QAS cavity disinfectant may be used for disinfecting cavities to be restored by amalgam or glass ionomer cements.

However, the use of cavity disinfectants that do not copolymerise with dentine adhesives may influence the bond strength of those dentine adhesives [31]. It is not known if the QAS cavity disinfectant has any adverse effect on dental pulp cells and dentine bonding after it is applied to acid-etched dentine. Accordingly, prior to clinical evaluation, the effect of QAS cavity disinfectant on the viability of human dental pulp cells, resin–dentine bond durability and nanoleakage should be characterised. Thus, the objectives of the present study were to evaluate the cytotoxicity of QAS on human dental pulp cells. The effect of dentine pre-treatment with different concentrations of the QAS cavity disinfectant on immediate, long-term bond strength and nanoleakage of simplified etch-and-rinse adhesives were also examined. The null hypotheses tested were that (i) there is no difference in cytotoxicity between QAS and CHX, (ii) pre-treatment of dentine with QAS does not affect the immediate dentine bond strength and (iii) pre-treatment of dentine with QAS has no effect in preventing degradation of the resin–dentine interface over time.

2. Materials and methods

2.1. Synthesis

The experimental versions of QAS cavity disinfectant examined in the present study were synthesised by sol–gel reaction between

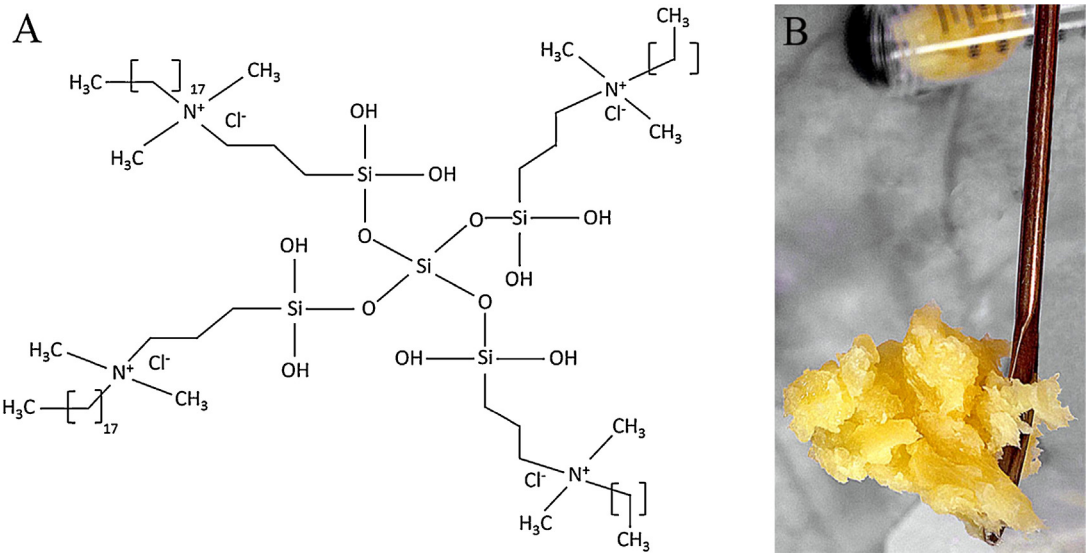

Fig. 1. Proposed chemical formula of the quaternary ammonium silane molecule K21. (For interpretation of the references to color in the text, the reader is referred to the web version of this article.)
Reproduced with permission from KHG fiteBac Technology, Marietta, GA, USA

1 mole of TEOS (Mw 208) and 4 moles of Et-SiQAC (Mw 538). In a typical synthesis, 2.08 g of TEOS was blended with 29.89 g of Et-SiQAC (72 wt% of Et-SiQAC dissolved in ethanol) and 5 mL of ethanol (to render the blend more homogeneous). Hydrolysis was initiated by the addition of 10.08 g of 0.02 M HCl-acidified water (pH 1.66, representing 3.5 times the stoichiometric molar concentration of water required, to ensure complete hydrolysis). Hydrolysis and condensation of the two ethoxysilanes was monitored by Fourier transform infrared spectroscopy [32].

Following completion of the hydrolysis reaction (approximately 3 h), a yellow solution mixture was obtained, the IR spectrum of which was characterised by the presence of silanol groups, ethanol and water [32]. The yellow solution mixture was then maintained at 80 °C for 6 h to remove as much as possible the reaction by-products (ethanol and water), until a pale yellow rubbery solid material was produced. This yellow solid (Fig. 1B) represented a partially-condensed form of the K21 molecule shown in Fig. 1A. Full condensation could be achieved by adding an alkali solution to raise the pH of the solution to 7.2, [32], wherein an ethanol-insoluble precipitate was progressively produced. This procedure was omitted during the synthesis phase and complete hydrolysis was subsequently achieved after the material was applied on dentine (to be reported in Section 2.3). The as-synthesised QAS solid was dissolved immediately in absolute ethanol to produce a 50% QAS solution. The 50% solution was further diluted with absolute ethanol to produce 2%, 5%, and 10% (w/v) QAS solutions. The three QAS solutions were stored in airtight vials and stored at 4 °C until use.

2.2. Microtensile bond strength and nanoleakage evaluation

2.2.1. Tooth preparation

One hundred and seventeen freshly extracted, sound human third molars (age range 21–34 years) that had been stored in chloramine T solution at 4 °C for no more than three months after extraction were used in the present study. The teeth were collected after the patient's informed consent was obtained under a protocol approved by the Institutional Review Board of The University of Hong Kong (UW 14-406). Sixty teeth were used for microtensile bond strength testing and twenty teeth for nanoleakage evaluation. Eighteen teeth were used to evaluate the effect of QAS condensation on etched dentine and the resin–dentine interface using scanning electron microscopy (SEM) and confocal laser scanning microscopy (CLSM). Nineteen teeth were used for cell viability assay.

The occlusal enamel was cut perpendicular to the longitudinal axis of each tooth with a slow-speed diamond-impregnated disc (Isomet, Buehler Ltd., Lake Bluff, IL, USA) under water cooling. The sectioning procedure exposed a flat mid-coronal dentine surface (1 mm below the dentinoenamel junction). The exposed dentine was polished wet with 180-grit silicon carbide paper to create a standardised smear layer. The specimens were ultrasonically rinsed in deionised water for 5 min.

2.2.2. Bonding procedures

The exposed dentine surface of each tooth was acid-etched with 32% phosphoric acid (Uni-Etch, Bisco Inc., Schaumburg, IL, USA) for 15 s, rinsed with deionised water for 15 s and kept visibly moist. The teeth were randomly divided into two groups according to the adhesive employed: Adper™ Single Bond 2 (SB, 3M ESPE, St. Paul, MN, USA) and Prime & Bond® NT™ (PB, Dentsply DeTrey, 78467 Konstanz, Germany). The compositions of the two simplified etch-and-rinse adhesives are shown in Table 1. The specimens from each adhesive group were blot-dried and further randomly assigned to one of the following five subgroups for dentine pre-treatment with 2% CHX or QAS cavity disinfectants ($N = 6$):

Table 1
Composition of dental adhesives tested in the present study.

Adhesive	Composition
Adper™ Single Bond 2	Bis-GMA HEMA Copolymer of acrylic and itaconic acids Glycerol 1,3-dimethacrylate Diurethane dimethacrylate Silane-treated silica Ethanol Water
Prime & Bond® NT™	Di- and trimethacrylate resins PENTA Nanofillers – amorphous silicon dioxide Photoinitiators Stabilizers Cetylamine hydrofluoride Acetone

Abbreviations: Bis-GMA, bisphenol A glycidyl dimethacrylate; HEMA, 2-hydroxyethyl methacrylate; PENTA, dipentaerythritol penta acrylate monophosphate.

Group 1: deionised water (control)
Group 2: 2% chlorhexidine (CHX, Millipore Sigma, St. Louis, MO, USA)
Group 3: 2% QAS
Group 4: 5% QAS
Group 5: 10% QAS

The etched dentine surfaces were pre-treated with the cavity disinfectants or deionised water using a sterilised micro brush. The solutions were left undisturbed for 20 s and then gently air-dried. The adhesives were applied to the dentine surface according to the manufacturers' instructions. Briefly, after applying two consecutive coats of adhesive to the demineralised dentine surface for 10 s with a fully saturated applicator; the coats were gently air-dried for 5 s to evaporate the solvent and light-cured at room temperature using a light-emitting diode curing unit (Elipar S10, 3M ESPE) for 15 s with an output intensity of 600 mW/cm^2. Resin composite build-ups were performed with a light-cured resin composite (Filtek Z250, 3M ESPE) in four 1-mm thick increments. Each increment was individually light-cured for 20 s. The bonded specimens were stored in distilled water at 37 °C for 24 h.

2.2.3. Microtensile bond strength

The bonded teeth were sectioned occluso-gingivally into 0.9 mm × 0.9 mm composite-dentine beams, according to the non-trimming version of the microtensile test. The beams were randomly assigned for microtensile bond strength (μTBS) evaluation after 24 h, 6 months or 12 months of storage in artificial saliva at 37 °C, following the protocol of Pashley et al. [9]. The artificial saliva contained (mmoles/L): $CaCl_2$ (0.7), $MgCl_2{\cdot}6H_2O$ (0.2), KH_2PO_4 (4.0), KCl (30), NaN_3 (0.3), and HEPES buffer. The storage medium was changed once every week.

At each time point, each beam was attached to a Bencor Multi-T device (Danville Engineering, San Ramon, CA, USA) with cyanoacrylate adhesive (Zapit, Dental Ventures of North America, Corona, CA, USA) and stressed to failure under tension with a universal testing machine (Model 4440, Instron, Inc., Canton, MA, USA) at a crosshead speed of 1 mm/min. After the test, the specimens were removed from the jig using a scalpel blade. The cross-sectional area of each specimen at the site of fracture was measured to the nearest 0.01 mm with a pair of digital callipers (Model CD-6BS; Mitutoyo, Tokyo, Japan). The μTBS values were expressed in MPa by dividing the force at debonding with the cross-sectional surface area.

2.2.4. Failure mode

After bond strength testing, the dentine side of the fractured beams were mounted on aluminium stubs, sputter-coated with gold/palladium and examined using a scanning electron microscope (SEM; Hitachi S-3400N, Hitachi High Technologies America, Inc., Schaumburg, IL, USA) operated at 15 kV to determine the mode of failure. The failure modes were classified as (i) adhesive failure, if the fracture site was within the adhesive, (ii) mixed failure, if the fracture site extended into either the resin composite or dentine, (iii) cohesive failure in resin composite and (iv) cohesive failure in dentine.

2.2.5. Nanoleakage

Twenty teeth ($N=2$) were used for interfacial nanoleakage evaluation for each time period tested. The teeth were pre-treated with the cavity disinfectants, bonded with the simplified etch-and-rinse adhesives and stored in artificial saliva at 37 °C for evaluation at 24 h or 12 months, as previously described for bond strength testing. At each time point, two coats of fast-drying nail varnish were applied 1 mm from the bonded interfaces. After drying of the nail varnish, the specimens were immersed in 50 wt% of ammoniacal silver nitrate solution (pH 9.5) according to the method by Tay et al. [33].

After 24 h, the specimens were rinsed and washed with deionised water for 5 min and placed in a photodeveloping solution (Kodak Professional Dektol Developer, Rochester, New York, USA) for 8 h under fluorescent light to facilitate reduction of diamine silver ions into metallic silver grains. The specimens were subsequently retrieved from the solution and polished with diamond pastes (6 μm, 3 μm, 1 μm; Buehler Ltd., Lake Bluff, IL, USA) using a polishing cloth. After the nail varnish was carefully removed, the specimens were ultrasonically cleaned for 10 min, air-dried and mounted on aluminium stubs. The specimens were then stored in a desiccator for 24 h. After carbon-coating, the specimens were examined using SEM at 15 kV in the back-scattered mode, focusing on the silver tracer expression along the resin–dentine interface. Forty images of the resin–dentine interfaces were obtained from each group at 500× magnification. The extent of silver deposition along the interface was evaluated by two examiners separately. The amount of silver uptake was scored on a scale of 0–4 using the classification method by Saboia et al. [34]: 0, no nanoleakage; 1, <25% nanoleakage; 2, 25 ≤ 50% nanoleakage; 3, 50 ≤ 75% nanoleakage, 4 > 75% nanoleakage.

2.3. QAS condensation

2.3.1. Scanning electron microscopy

Because condensation of acid-catalysed, hydrolysed SiQAC occurs after its pH was adjusted to 7.0 [32,35], it was anticipated that neutralisation of the QAS solution by the buffering action of dentine [36] would lead to condensation of a 3-D network of QAS within the dentinal tubules. Accordingly, the exposed dentine surfaces of 4 teeth ($N=2$) were acid-etched with 32% phosphoric acid (Uni-Etch, Bisco Inc.) for 15 s, rinsed with deionised water for 15 s and kept visibly moist. Two percent QAS or 10% QAS was applied to the acid-etched dentine for 20 s. Following evaporation of the ethanol solvent from the QAS solution, no dentine adhesive was applied to the QAS-treated dentine. Whilst this procedure deviated from the dentine bonding procedures described in subsequent sections, the objective was to prevent resin tags produced by a polymerised dentine adhesive from interfering with the identification of condensed QAS within the dentinal tubules. Two parallel grooves were made on external surfaces in mesiodistal direction of each tooth to facilitate split-fracture. Final separation was made using chisel and hammer. The specimens were mounted on aluminium stubs with double-sided conductive tape, sputter-coated with gold/palladium and examined using SEM.

The remaining four teeth ($N=2$) were similarly prepared and bonded with SB and PB following the application of 2% or 10% QAS. The bonded interfaces were similarly evaluated using SEM. The bonded specimens were stored in water for 24 h and split-fractured, in the mesiodistal direction. The splitted fragments were mounted on aluminium stubs, sputtered with gold/palladium and examined with SEM at 10 kV.

2.3.2. Confocal laser scanning microscopy

Non-destructive identification of QAS within the resin–dentine interface was performed using 0.1 wt % aqueous solution of sodium fluorescein (46960 Bioreagent, Millipore Sigma) [37]. The fluoroscein was selected to trace the location of QAS within the hybrid layer and along the dentinal tubules. Two teeth were bonded with SB following the application of 2% QAS as previously described. The bonded specimens were sectioned into 0.5 mm thick mesio-distal slabs using a slow-speed water-cooled diamond saw. Two slabs from the centre of each tooth were selected. The slabs were slightly polished with wet 1200-grit silicon carbide paper for 30 s, ultrasonicated for 10 min and immersed in 0.1 wt% fluorescein for 24 h. The slabs were then rinsed with deionised water and examined using confocal laser scanning microscope (CLSM; Leica Fluoview FV 1000, Olympus, Tokyo, Japan) equipped with a 60×/1.4 NA oil immersion lens using 488 nm argon/helium and a 633 nm krypton ion laser illumination both in reflection and fluorescence modes. Reflected and fluorescence signals were detected with a photomultiplier tube to a depth of 20 μm and then converted to single-projection images for better visualization and qualitative analysis [38,39]. Two images were obtained for each slab and the images representing the most common features regarding QAS location along the bonded interfaces.

2.4. Cell viability

2.4.1. Preparation of dentine discs

Nine teeth were used to compare the cytotoxic effects of 2% QAS-treated, 2% CHX-treated and untreated (control) dentine specimens. The roots were removed using the low-speed, water-cooled diamond saw, and the occlusal enamel of each crown segment was cut to expose dentine. A 0.5 mm thick dentine disc was prepared from the mid-coronal dentine of each tooth and the enamel was removed from the sides to produce square-shaped dentine discs. A final disc thickness of about 0.4 mm was achieved by grinding the occlusal side with wet 320-grit silicon carbide paper. The thickness was evaluated using a pair of digital callipers with a precision of 0.01 mm.

2.4.2. Dentine permeability

Dentine permeability was evaluated to enable a homogenous distribution of the dentine discs into three groups ($N=3$). Dentine permeability was determined by removing the smear layer from both sides of the discs by applying 0.5 M ethylenediamine tetra acetic acid (pH 7.4) for 60 s. After rinsing, the discs were placed in a diffusion chamber connected to 180 cm column of water for 5 min. The movement of a micro-bubble introduced through a metallic cannula was recorded for 1 min. Calculation of the hydraulic conductance (Lp) of dentine was based on a mathematical equation "$Lp = Jv/At\ (P)$", where Lp = hydraulic conductance in μL cm^{-2}, min^{-1}, H_2O^{-1}, Jv = fluid flow in μL min, A = surface area of the dentine in cm^2, t = time, and P = hydrostatic pressure applied in cm H_2O [40]. The dentine discs were then allocated into groups so that the mean hydraulic conductance was not statistically different among the three groups (one-way analysis of variance, $p > 0.05$). An area of 0.28 cm^2 was standardised using a metallic ring on the

dentine disc, and a fresh smear layer was created using 600-grit silicon carbide paper for 10 s.

2.4.3. Culture of human dental pulpal cell line

Viability of human dental pulpal cells (hDPCs) was measured by monitoring their metabolic activity using 3-(4,5-dimethylthiazolyl-2)-2,5-diphenyltetrazolium bromide (MTT) assay. Ten human third molars were collected with written informed consent from patients (20–34 years of age) who have been planned to have those teeth extracted for orthodontic or therapeutic reasons. The teeth were mechanically fractured with surgical chisels longitudinally. Dental pulp tissues were obtained using forceps. The pulp tissues were stored in alpha Minimal Essential Medium (GIBCO Invitrogen Corp., Paisley, Scotland, UK), which had previously been supplemented with foetal-calf serum (FCS), penicillin (100 U/mL), streptomycin (100 mg/mL) and glutamine (2 mmol/L). The pulpal tissues were minced and cultured in 6-well plates for two weeks at 37 °C in a humidified atmosphere containing 5% CO_2 and 95% atmospheric air. The medium was changed twice a week.

The cultured cells were expanded to 4× dilution after achieving 80% confluency. Cells sub-cultured to the sixth or seventh passages were utilised for the experiment. The cells (3×10^4) were seeded on the pulpal side of the dentine discs (0.28 cm^2) in 24-well plates in an incubator with 5% CO_2 and 95% air at 37 °C. The discs were transferred back to the same wells to receive the QAS and CHX treatments.

2.4.4. Application of QAS and CHX

The occlusal side of the dentine disc was etched using 35% phosphoric acid (Scotchbond etchant, 3M ESPE) for 15 s, rinsed with deionised water and blot-dried using lint-free tissues. Two percent QAS was applied onto the occlusal side of the dentine discs using a sterile microbrush for 20 s, followed by blot-drying. Likewise, 2% CHX was applied, while no pre-treatment was performed in the control group. After treatment with the cavity disinfectants, the dentine discs were returned to the CO_2 incubator for an additional 24 h.

2.4.5. MTT assay

The hDPCs cultured on dentine discs were retrieved by washing with phosphate buffered saline and incubating with 500 μg/mL MTT for 4 h at 37 °C. The insoluble formazan produced by the cells was dissolved in 400 μL acidified isopropanol (0.04 N HCl). Three 100 μL aliquots of the dissolved formazan supernatants were transferred to a 96-well plate and cell viability was evaluated by spectrophotometry at 570 nm using a microplate reader (Thermo Plate, Nanshan District, Shenzhen, Guangdong, China). Twelve replicates were performed for each experimental group and the results were expressed as percentages of the MTT activity of hDPSCs grown on culture plates, which was normalised to present 100% cell viability.

2.5. Statistical analysis

The bond strength data was expressed as means ± standard deviations and analysed using a statistical package (SigmaStat Version 20, SPSS, Chicago, IL, USA). Because the bond strength values were normally distributed (Shapiro–Wilk test) and homoscedastic (modified Levene test), the data were analysed by three-way analysis of variance (ANOVA) to examine the effects of "disinfectants", "adhesives" and "time", and the interaction of those factors on microtensile bond strength. Post hoc multiple comparisons were performed using Tukey's test. For nanoleakage evaluation, the inter-observer reproducibility and the intra-observer reproducibility were evaluated using weighted Kappa (κ_w) statistic. The nanoleakage scores were treated as ordinal data. The Cochran-Mantel-Haenszel (CMH) method was used to test significant differences of the five treatment groups (control, 2% CHX, 2% QAS, 5% QAS, 10% QAS) at the two time periods (24 h vs. 12 months). A significant level of $\alpha = 0.05$ was used. Data for cell viability assay were analysed using one-way ANOVA followed by Tukey's multiple comparison procedures.

3. Results

3.1. Microtensile bond strength

Microtensile bond strength for each group at 24 h, 6 and 12 months are summarised in Table 2. Three-way ANOVA showed that the factors, "disinfectants" ($p < 0.001$, $F = 41.45$) and "time" ($p < 0.001$, $F = 9.97$) significantly affected the bond strength to dentine; while the factor "adhesive" did not significantly affect bond strength ($p = 0.203$, $F = 1.621$). The interactions between "time" and "disinfectant" ($p < 0.001$, $F = 9.38$) as well as "adhesive" and "disinfectant" ($p < 0.01$, $F = 3.40$) were significant; while the interaction between "time" and "adhesive" was not significant ($p = 0.052$, $F = 2.97$). The interaction of the three factors was not significant ($p = 0.104$, $F = 1.66$). That is, the change in bond strength during storage was independent of the disinfectant and adhesive.

At baseline, bond strength reduced significantly as the concentration of QAS was increased to 10% in both adhesives ($p < 0.05$). For PB, no significant difference in bond strength was found among 2% CHX, 2% QAS, 5% QAS and the control ($p > 0.05$). For SB, no significant difference in bond strength was found among 2% CHX, 2% QAS and the control ($p > 0.05$). The use of 2% QAS before

Table 2
Microtensile bond strength after 24 h, 6 months and 12 months of ageing in artificial saliva.

Adhesives	Cavity disinfectants	24 h	6 months	12 months
Adper™ Single Bond 2	Control	33.9 ± 7.8 A α †	29.0 ± 6.0 b β ‡	23.9 ± 3.9 III γ ‡
	2% CHX	32.5 ± 6.5 A α †	31.2 ± 5.1 a α †	29.9 ± 4.7 I α †
	2% QAS	30.7 ± 6.0 A α †	33.2 ± 5.1 a α †	31.8 ± 5.6 I α †
	5% QAS	28.4 ± 6. 6 B ß ‡	27.2 ± 7.8 b β †	30.1 ± 4.3 I α †
	10% QAS	27.7 ± 5.1 B α †	24.8 ± 4.2 c β ‡	21.3 ± 3.9 III β ‡
Prime & Bond® NT™	Control	35.4 ± 7.5 A α †	31.1 ± 5.3 a α †	28.0 ± 4.2 II β †
	2% CHX	33.1 ± 4.2 A α †	32.0 ± 7.0 a α †	30.2 ± 5.7 I α †
	2% QAS	31.5 ± 5.2 A α †	32.2 ± 6.9 a α †	32.9 ± 5.1 I α †
	5% QAS	30.2 ± 8.3 A α †	28.5 ± 5.4 b β †	26.8 ± 4.1 II β ‡
	10% QAS	29.1 ± 8.8 B α †	27.1 ± 6.3 b α †	25.1 ± 5.2 III β †

Values are means ± standard deviations, in MPa. Uppercase letters/lowercase letters/Roman numerals represent differences between each column. Symbols (α, β and γ) represent differences between rows. Symbols († and ‡) represent differences between adhesives. Groups identified by different superscripts are significantly different.

adhesive application did not adversely affect the immediate bond strength of either adhesive.

In the absence of pre-treatment, the bond strength of the control group of both adhesives dropped significantly after 12 months ($p < 0.05$). By contrast, bond strength did not significantly decline with the use of 2% QAS for both PB and SB after 12 months of ageing in artificial saliva. Similarly, no significant change in bond strength was observed in the 2% CHX groups for both adhesives after 12 months, when compared to 24 h. No statistically significant difference was observed between the 2% CHX and 2% QAS groups of the two adhesives at 24 h, 6 months or 12 months.

3.2. Failure mode distribution

Table 3 shows the percentage distribution of failure modes of the debonded dentine specimens in the two adhesives groups at 24 h and 12 months, respectively. In general, low microtensile bond strength values were associated with a higher tendency to fail within the adhesive (i.e. adhesive failure). The failure modes determined for all test specimens showed predominant mixed failures. Nevertheless, a clear prevalence of adhesive failures was observed for the control group as well as the 5% and 10% QAS groups in both adhesives after12 months of ageing in artificial saliva.

3.3. Nanoleakage

The weighed Kappa for intra-observer reproducibility exceeded the 0.70 cut off, with a mean of 0.86, indicating almost perfect reproducibility [41]; while the mean weighted Kappa (κ_w) for inter-observer reproducibility was 0.80, showing substantial reproducibility [41]. The distribution of nanoleakage scores of the five different groups of both adhesives at baseline and 12 months is shown in Table 4. The Cochran–Mantel–Haenszel method was used to test for significant difference among each pair of treatment groups at baseline and 12 months. For PB, no significant difference in nanoleakage score was found in 2% QAS group between baseline and 12 months ($p > 0.05$); while significant difference in nanoleakage score was observed in control, 2% CHX, 5% and 10% QAS groups ($p < 0.05$). Conversely, for SB, no significant difference in nanoleakage score was found in 2% and 10% QAS groups between baseline and 12 months ($p > 0.05$); while significant difference in nanoleakage score was observed in control, 2% CHX and 5% QAS groups ($p < 0.01$).

Table 4
Distribution of the nanoleakage scores of the five treatment groups at baseline and 12 months.

Adhesives	Cavity disinfectants	Nanoleakage score					
Prime & Bond® NT™		**0**	**1**	**2**	**3**	**4**	*p* Value
Baseline	Control	8	11	10	3	8	0.049
	2% CHX	4	8	10	12	6	0.021
	2% QAS	6	12	8	10	4	0.671
	5% QAS	0	10	14	12	4	0.042
	10% QAS	0	12	10	8	10	0.004
12 months	Control	0	2	16	16	6	
	2% CHX	0	4	20	16	0	
	2% QAS	0	12	20	8	0	
	5% QAS	0	8	8	12	12	
	10% QAS	0	0	4	20	16	
Adper™ Single Bond 2							
Baseline	Control	4	14	8	10	4	0.000
	2% CHX	2	12	8	10	8	0.003
	2% QAS	4	14	12	4	6	0.355
	5% QAS	0	10	10	8	12	0.001
	10% QAS	0	4	6	14	16	0.064
12 months	Control	0	0	16	8	16	
	2% CHX	0	0	4	24	12	
	2% QAS	4	4	16	12	4	
	5% QAS	0	0	8	16	16	
	10% QAS	0	0	4	12	24	

Extent of interfacial nanoleakage 0: 0%; 1: <25%; 2: 25–50%; 3: 50–75%; 4: >75%. Cochran-Mantel-Haenszel test ($2 \times 2 \times 5$).

Table 3
Percentage distribution of failure mode after 24 h and 12 months of ageing in artificial saliva.

Group	Cavity disinfectants	Failure mode	Adper™ Single Bond 2		Prime & Bond® NT™	
			24 h	12 months	24 h	12 months
I	Control	A	26	37	19	23
		M	37	29	45	51
		CD	21	13	24	7
		CC	16	21	12	19
II	2% CHX	A	27	18	28	10
		M	37	54	52	45
		CD	17	11	12	18
		CC	19	17	8	27
III	2% QAS	A	12	9	11	19
		M	40	56	48	45
		CD	23	16	24	13
		CC	25	19	17	23
IV	5% QAS	A	27	31	18	31
		M	48	33	56	43
		CD	14	9	17	11
		CC	11	33	9	15
V	10% QAS	A	28	38	26	41
		M	44	29	49	38
		CD	11	7	21	13
		CC	17	26	4	10

Failure mode: A, adhesive; CD, cohesive failure in dentine; CC, cohesive failure in resin composite; M, mixed failure.

3.4. SEM and CLSM of QAS condensation

Scanning electron microscopy images of etched dentine after the application of 2% and 10% QAS solutions (without adhesive) are shown in Fig. 2A and B, respectively. After QAS application and air-drying to evaporate the ethanol solvent, both 2% and 10% QAS formed a crust on the surface of the acid-etched dentine. Without the use of a dentine adhesive, the QAS did not infiltrate well into demineralised collagen matrix. As a result, denuded, shrunken collagen could be identified from the poorly-infiltrated collagen matrix. However, following the application of PB to 2% QAS-treated acid-etched dentine, a hybrid layer was produced by the adhesive. The crust produced by 2% QAS was completely dissolved by the adhesive solvent and could no longer be identified from the resin–dentine interface (Fig. 2C). The PB adhesive together with the dissolved 2% QAS infiltrated the underlying demineralised dentine and dentinal tubules (Fig. 2D). Resin tags could be identified within the dentinal tubules and their lateral ramifications. Unlike the use of 2% QAS, incomplete infiltration of PB into the 10% QAS-treated dentine resulted in a visually identifiable porous hybrid layer (Fig. 2E). Likewise, a visually intact resin-infiltrated hybrid layer was observed after the application of SB to 2% QAS-treated dentine (Fig. 2F). With the use of 10% QAS, only short resin tags were formed with SB-bonded dentine (Fig. 2G). Condensation of the QAS could be seen as spherical bodies within the dentinal tubules, which is indicative of a phase separation phenomenon in the presence of the water present inside the tubules (Fig. 2H). A merged CLSM image of a representative specimen from the 2% QAS-SB group is shown in Fig. 2I. The location of the QAS could be identified as it was pre-stained with sodium fluorescein. Although both adhesive and dentine also exhibited mild autofluorescence, the highly fluorescent, the top 50 μm of dentine was devoid of the highly-fluorescent QAS. This region corresponded to the unstained resin tags produced by the dentine adhesive.

3.5. MTT assay

The viability of hDPCs cultured on dentine discs after exposure to the cavity disinfectants or the control are collectively represented in Fig. 3. Normalised cell viability was in the order (from the lowest to the highest): 2% CHX (19.3%) < 2% QAS (55.1%) < the negative control (80.2%) [$p < 0.05$].

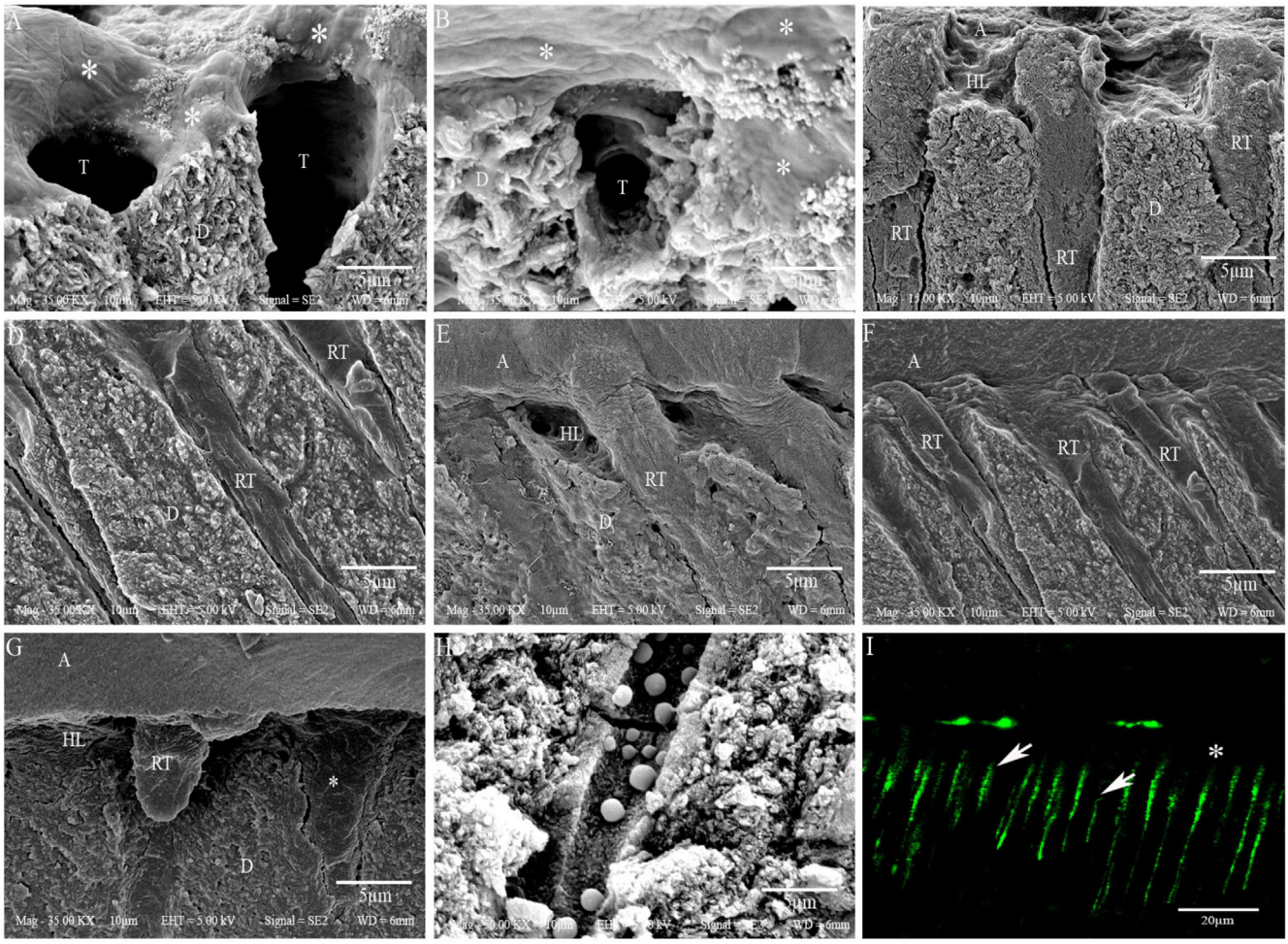

Fig. 2. Representative SEM images of etched dentine following application of (A) 2% QAS (*) and (B) 10% QAS (*). The QAS did not infiltrate well into demineralized collagen matrix and dentinal tubules, but formed a crust on the surface of acid-etched dentine. (C) After application of Prime & Bond® NT™ (PB) adhesive to 2% QAS-treated dentine, the QAS layer was completely dissolved, allowing infiltration of the adhesive into demineralized dentine to form a hybrid layer. (D) Regular resin tags were formed within the tubules in the 2% QAS/PB group. (E) Incomplete penetration of PB into 10% QAS-treated dentine, resulting in a porous hybrid layer. (F) Hybrid layer and resin tags formation in 2% QAS/Adper™ Single Bond 2 (SB) group. (G) Minimal resin penetration and resin tags formation in the 10% QAS/SB group. (H) The 2% QAS were pushed down by the adhesive resin, phase separated in the presence of water within the dentinal tubules and produced spherical structures further down the tubules. (I) CLSM merged image after application of 2% QAS and SB, with sodium fluorescein staining of the QAS. The adhesive and dentine (asterisk) exhibited slight background auto fluorescence. The QAS was displaced by the adhesive down the dentinal tubules (arrows). The top 50 μm of dentine did not contain stained tags. This area corresponds to resin tags formed by the dentine adhesive. (A: adhesive; D: demineralised dentine; HL: hybrid layer; T: dentinal tubules).

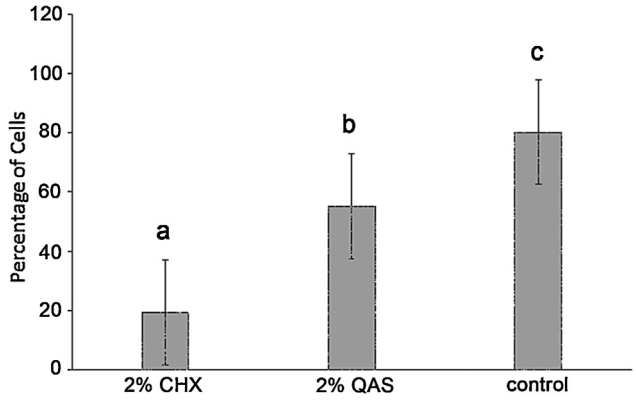

Fig. 3. Cell viability as measured by absorbance of human dental pulp cells after exposure to 2% QAS, 2% CHX and no treatment (control) for 24 h. Groups identified by different lower case letters are significantly different ($p < 0.05$).

4. Discussion

In the era of minimal intervention dentistry that emphasizes selective and conservative removal of tooth structure, caries-infected tissues may be deliberately retained along the pulpal floor to preserve pulp vitality [42]. Residual bacterial species such as *S. mutans* may proliferate in the smear layer and dentinal tubules, causing secondary caries and eventually resulting in irreversible pulpitis, pulpal necrosis and apical periodontitis [43]. Hence, there is a demand for cavity disinfectants that are applied prior to the insertion of non-antimicrobial restorative materials [5]. An ideal cavity disinfectant should possess strong antimicrobial activity, whilst having minimal adverse effects on adhesive bonding. The present work represents a proof-of-concept strategy to circumvent the problem of secondary caries proliferation and preservation of bond durability of simplified etch-and-rinse adhesives over time.

Similar to CHX, QAS are cationic antimicrobials commonly used for industrial and pharmaceutical purposes. Because of their surfactant properties, organofunctional silanes form a protective antimicrobial film after reacting with the surface of materials. This surfactant activity may result in reduction of microbial adherence to circumvent or slow down biofilm formation [44]. Due to the presence of long chain lipophilic alkyl chains, the action of quaternary ammonium compounds against microbes is strong and rapid [45]. Sol–gel synthesis provides a facile method for synthesising organosilicates under mild conditions. The use of a tetrafunctional organosilane as anchoring unit for the antimicrobial trialkoxysilane molecules enables a three-dimensional network to be formed once condensation is brought to completion within the dentinal substrate [46]. This minimizes the possibility for individual Et-SiQAC molecules to leach out of the resin–dentine interface, and provides considerably longer antimicrobial activity.

Antimicrobials used as cavity disinfectants should be biocompatible because of the potential of reaching the dental pulp when these materials are applied to deep, smear layer-depleted dentine. Chlorhexidine is commonly used as a cavity disinfectant and a 2% aqueous solution of chlorhexidine has been considered highly biocompatible [47]. The present study compared the ex vivo biocompatibility of QAS with CHX after these solutions were in contact with hDPCs for 24 h. The cavity disinfectants were applied on 0.4 mm thick dentine discs, a standard screening model for evaluation of transdentinal cytotoxicity, to better simulate the clinical situation [48]. Results from the cytotoxicity assay indicated that a higher percentage of hDPCs remained viable after they came in contact with 2% QAS, compared to 2% CHX. Hence, the first null hypothesis that there is no difference in cytotoxicity between QAS and CHX has to be rejected. This renders QAS a safer cavity disinfectant than CHX for incorporation into the resin–dentine bonding protocol. Further studies are required to elucidate the mechanisms responsible for the more favourable biocompatibility of QAS.

For both SB and PB, tensile bond strengths from the groups pre-treated with 2% CHX or 2% QAS were not significantly different from the control group at baseline. For PB, bond strength decreased significantly when QAS concentration was higher than 5%. Similar decrease in bond strength was observed in SB, when the QAS concentration was higher than 2%. Hence, the second null hypothesis that pre-treatment of dentine with QAS would not affect immediate dentine bond strength has to be partially rejected.

The bond strength results in the control group after 12 months of ageing in artificial saliva are in accordance with previous studies, showing the gradual deterioration of the bonded interface as a result of swelling and plasticising of the adhesive resin as well as degradation of the demineralised collagen following ageing [49,50]. Pre-treatment of acid-etched dentine with 2% CHX and 2% QAS before application of both adhesives resulted in the preservation of tensile bond strength after ageing in artificial saliva. Conversely, tensile bond strength obtained from the 5% and 10% QAS groups fell significantly, while nanoleakage increased significantly over time. %. No significant difference in nanoleakage score was observed in the 2% QAS group of both adhesives between baseline and 12 months. However, nanoleakage was significantly higher in the control, 2% CHX and 5% QAS groups of both adhesives at 12 months. Hence, the third hypothesis that pre-treatment of dentine with QAS does not prevent degradation of the bonded interface over time has to be partially rejected.

Organofunctional silanes are stable, non-polar and hydrophobic structures, which are mainly used for surface modification and adhesion promotion. When silane primer contact dentine before adhesive application, the primer does not chemically bond to dentine. It has been shown that the unreacted silane monomer co-polymerises with dental adhesives with no adverse effect on resin–dentine bond strength [51]. Non-polymerisable silane primers, which were mixed with 2-hydroxyethyl methacrylate (HEMA), have been shown to significantly increase dentine bond strength due to the formation of a more durable hybrid layer [52]. This may be attributed to the presence of bridged organosiloxane groups in the molecular backbone of QAS, which has affinity for the resinous adhesive [53]. Furthermore, occlusion of dentinal tubules by condensed QAS provides the optimal conditions for reduction of dentine permeability and the establishment of a more hydrophobic bonded interface.

Because organofunctional silanes consist of long hydrocarbon chains, pre-treatment of acid-etched dentine with QAS would increase surface hydrophobicity by changing the surface energy of the dentine substrate. Increased surface energy would in turn result in better wettability of the demineralised dentine surface and enhanced infiltration of the adhesive resin monomers [54]. Organofunctional silanes are also water scavengers, which react with water molecules, hydrolyzing alkoxy groups attached to the silanes and converting them to alcohol molecules [55]. The incorporation of hydrophobic QAS into the bonded interfaces resulted in the formation of a more hydrophobic and durable hybrid layer. Reduction of residual water within the demineralised dentine matrix is likely to facilitate infiltration and polymerisation of the adhesive resins, resulting in better encapsulation of the collagen fibrils, increased mechanical properties of the adhesive layer and improved bond durability [56,57].

Reduction in bond strength observed in 5% and 10% QAS groups may be attributed to several reasons. First, higher concentration of QAS may form a condensed layer on the demineralised dentine surface, which serve as stress-raisers initiating micro-fractures and

cracks, causing detachment of the overlying resin composite. This is supported by the nanoleakage results, where extensive accumulation of silver deposits was observed within the hybrid layers and at the resin–dentine bonded interface when the QAS concentration was higher than 10%. Second, the presence of a higher concentration of QAS in the demineralised dentine may have modified the polymerisation of the adhesive resin of SB and PB, thereby affecting their final degree of conversion, physical and mechanical properties. Insufficient polymerisation of the adhesive resin may result in a weakened hybrid layer, decreasing the bond strength and longevity over time [58–60]. Further studies are required to investigate the effect of different concentrations of QAS on the degree of conversion and modulus of elasticity of adhesive resins. In summary, the results of the present study show that 2% QAS is an alternative cavity disinfectant to 2% CHX, with minimal effects on the immediate bond strength of the simplified etch-and-rinse adhesives, and effectively preserves the stability of the hybrid layer and bonded interface over time.

5. Conclusion

The present study confirmed that the experimental cavity disinfectant containing 2% quaternary ammonium silane preserves the viability of human dental pulp cells compared to 2% chlorhexidine. The 2% quaternary ammonium silane cavity disinfectant shows no adverse effect on the immediate bond strength of both simplified etch-and-rinse adhesives and maintain the stability of resin–dentine bonds produced by those adhesives over time.

Acknowledgement

The quaternary ammonium silane cavity disinfectant used in this study was generously supplied by KHG fiteBac Technology, Marietta, GA, USA.

References

[1] J.L. Ferracane, Resin-based composite performance: are there some things we can't predict? Dent. Mater. 29 (2013) 51–58.
[2] E.A. Kidd, O. Fejerskov, What constitutes dental caries? Histopathology of carious enamel and dentin related to the action of cariogenic biofilms, J. Dent. Res. 83 (2004) Spec No C:C35–38.
[3] R. Hickel, J. Manhart, Longevity of restorations in posterior teeth and reasons for failure, J. Adhes. Dent. 3 (2001) 45–64.
[4] R.L. Sakaguchi, Review of the current status and challenges for dental posterior restorative composites: clinical, chemistry, and physical behaviour considerations. Summary of discussion from the Portland Composites Symposium (POCOS) June 17–19, 2004, Oregon Health and Science University, Portland, Oregon, Dent. Mater. 21 (2005) 3–6.
[5] J.C. Meiers, J.C. Kresin, Cavity disinfectants and dentin bonding, Oper. Dent. 21 (1996) 153–159.
[6] F.L. De Castro, M.F. de Andrade, S.L. Duarte Júnior, L.G. Vaz, F.J. Ahid, Effect of 2% chlorhexidine on microtensile bond strength of composite to dentin, J. Adhes. Dent. 5 (2003) 129–138.
[7] S. Twetman, Antimicrobials in future caries control? A review with special reference to chlorhexidine treatment, Caries Res. 38 (2004) 223–229.
[8] R.I. Gendron, D. Grenier, T. Sorsa, D. Mayrand, Inhibition of the activities of matrix metalloproteinases 2, 8, and 9 by chlorhexidine, Clin. Diag. Lab. Immun. 6 (1999) 437–439.
[9] D.H. Pashley, F.R. Tay, C.K. Yiu, M. Hashimoto, L. Breschi, R.M. Carvalho, S. Ito, Collagen degradation by host-derived enzymes during aging, J. Dent. Res. 83 (2004) 216–221.
[10] P.M. Scaffa, C.M. Vidal, N. Barros, T.F. Gesteira, A.K. Carmona, L. Breschi, D.H. Pashley, L. Tjäderhane, I.L. Tersariol, F.D. Nascimento, M.R. Carrilho, Chlorhexidine inhibits the activity of dental cysteine cathepsins, J. Dent. Res. 91 (2012) 420–425.
[11] L. Tjäderhane, F.D. Nascimento, L. Breschi, A. Mazzoni, I.L. Tersariol, S. Geraldeli, A. Tezvergil-Mutluay, M. Carrilho, R.M. Carvalho, F.R. Tay, D.H. Pashley, Strategies to prevent hydrolytic degradation of the hybrid layer – a review, Dent. Mater. 29 (2013) 999–1011.
[12] L. García-Caballero, V. Quintas, I. Prada-López, J. Seoane, N. Donos, I. Tomás, Chlorhexidine substantivity on salivary flora and plaque-like biofilm: an in situ model, PLOS ONE 8 (2013) e83522.
[13] J. Kim, T. Uchiyama, M. Carrilho, K.A. Agee, A. Mazzoni, L. Breschi, R.M. Carvalho, L. Tjaderhane, S. Looney, C. Wimmer, A. Tezvergil-Mutluay, F.R. Tay, D.H. Pashley, Chlorhexidine binding to mineralized versus demineralized dentin powder, Dent. Mater. 26 (2010) 771–778.
[14] F.T. Sadek, R.R. Braga, A. Muench, Y. Liu, D.H. Pashley, F.R. Tay, Ethanol wet-bonding challenges current anti-degradation strategy, J. Dent. Res. 89 (2010) 1499–1504.
[15] F.C.I. Lessa, I. Nogueira, C. Huck, J. Hebling, C.A. Costa, Transdentinal cytotoxic effects of different concentrations of chlorhexidine gel applied on acid-conditioned dentin substrate, J. Biomed. Mater. Res. B: Appl. Biomater. 92 (2010) 40–47.
[16] B. Gottenbos, H.C. van der Mei, F. Klatter, P. Nieuwenhuis, H.J. Busscher, In vitro and in vivo antimicrobial activity of covalently coupled quaternary ammonium silane coatings on silicone rubber, Biomaterials 23 (2002) 1417–1423.
[17] J.J. Oosterhof, K.J. Buijssen, H.J. Busscher, B.F. van der Laan, H.C. van der Mei, Effects of quaternary ammonium silane coatings on mixed fungal and bacterial biofilms on tracheoesophageal shunt prosthesis, Appl. Environ. Microbiol. 7 (2006) 3673–3677.
[18] J.W.M. Yuen, J.K.Y. Yung, Medical implications of antimicrobial coating polymers – organosilicon quaternary ammonium chloride, Mod. Chem. Appl. 1 (2013) 107, doi:http://dx.doi.org/10.4172/2329-6798.1000107.
[19] L. Song, R.H. Baney, Antibacterial evaluation of cotton textile treated by trialkoxysilane compounds with antimicrobial moiety, Text. Res. J. 81 (2010) 504–511.
[20] Reregistration eligibility decision for trimethoxysilyl quaternary ammonium chloride compounds. United States Environmental Protection Agency 2007; EPA 739-R-07-007.
[21] A.J. Isquith, E.A. Abbott, P.A. Walters, Surface-bonded antimicrobial activity of an organosilicon quaternary ammonium chloride, Appl. Microbiol. 24 (1972) 859–863.
[22] B. Ahlström, R.A. Thompson, L. Edebo, Loss pf bactericidal capacity of long-chain quaternary ammonium compounds with protein at lowered temperature, APMIS 107 (1999) 606–614.
[23] S.Q. Gong, L.N. Niu, L.K. Kemp, C.K. Yiu, H. Ryou, Y.P. Qi, J.D. Blizzard, S. Nikonov, M.G. Brackett, R.L. Messer, C.D. Wu, J. Mao, L. Bryan Brister, F.A. Rueggeberg, D. D. Arola, D.H. Pashley, F.R. Tay, Quaternary ammonium silane-functionalized, methacrylate resin composition with antimicrobial activities and self-repair potential, Acta. Biomater. 8 (2012) 3270–3282.
[24] S.Q. Gong, J. Epasinghe, F.A. Rueggeberg, L.N. Niu, D. Mettenberg, C.K. Yiu, J.D. Blizzard, C.D. Wu, J. Mao, C.L. Drisko, D.H. Pashley, F.R. Tay, An ORMOSIL-containing orthodontic acrylic resin with concomitant improvements in antimicrobial and fracture toughness properties, PLoS ONE 7 (2012) e42355.
[25] S.Q. Gong, D.J. Epasinghe, B. Zhou, L.N. Niu, K.A. Kimmerling, F.A. Rueggeberg, C.K. Yiu, J. Mao, D.H. Pashley, F.R. Tay, Effect of water-aging on the antimicrobial activities of an ORMOSIL-containing orthodontic acrylic resin, Acta. Biomater. 9 (2013) 6964–6973.
[26] S.Q. Gong, Z.B. Huang, W. Shi, B. Ma, F.R. Tay, B. Zhou, In vitro evaluation of antibacterial effect of AH Plus incorporated with quaternary ammonium epoxy silicate against *Enterococcus faecalis*, J. Endod. 40 (2014) 1611–1615.
[27] W. Zhang, X.J. Luo, L.N. Niu, S.Y. Liu, W.C. Zhu, J. Epasinghe, L. Chen, C. Huang, J. Mao, One-pot synthesis of antibacterial monomers with dual biocidal modes, J. Dent. 42 (2014) 1078–1095.
[28] S.Q. Gong, D.J. Epasinghe, W. Zhang, B. Zhou, L.N. Niu, H. Ryou, A.A. Eid, A. Frasseto, C.K.Y. Yiu, D.D. Arola, J. Mao, Synthesis of antimicrobial silsesquioxane–silica hybrids by hydrolytic co-condensation of alkoxysilanes, Polym. Chem. 5 (2014) 454–462.
[29] V. Thompson, R.G. Craig, F.A. Curro, W.S. Green, J.A. Ship, Treatment of deep carious lesions by complete excavation or partial removal: a critical review, J. Am. Dent. Assoc. 139 (2008) 705–712.
[30] S. Sauro, D.H. Pashley, Strategies to destabilize dentine-bonded interfaces through remineralising operative procedures – state of the art, Int. J. Adhes. Adhes. 69 (2016) 39–57.
[31] C. Celik, Y. Ozel, B. Bagis, S. Erkut, Effect of laser irradiation and cavity disinfectant application on the microtensile bond strength of different adhesive systems, Photomed. Laser Surg. 28 (2010) 267–272.
[32] D.E. Leyden, R.S. Shreedhara Murthy, J.P. Blitz, J.B. Atwater, A. Rachetti, Reflectance FTIR investigations of the reactions of silanes on silica surfaces, Microchim. Acta (1998) 53–56.
[33] F.R. Tay, D.H. Pashley, M. Yoshiyama, Two modes of nanoleakage expression in single-step adhesives, J. Dent. Res. 81 (2002) 472–476.
[34] V.P. Saboia, F. Nato, A. Mazzoni, G. Orsini, A. Putignano, M. Giannini, Adhesion of a two-step etch-and-rinse adhesive on collagen-depleted dentin, J. Adhes. Dent. 10 (2008) 419–422.
[35] D.R. Leyden, J.B. Atwater, Hydrolysis and condensation of alkoxysilanes investigated by internal reflection FTIR spectroscopy, J. Adhes. Sci. Technol. 10 (1991) 815–829.
[36] J. Camps, D.H. Pashley, Buffering action of human dentin in vitro, J. Adhes. Dent. 2 (2000) 39–50.
[37] H. Zhou, F. Li, M.D. Weir, H.H. Xu, Dental plaque microcosm response to bonding agents containing quaternary ammonium methacrylates with different chain lengths and charge densities, J. Dent. 41 (2013) 1122–1231.
[38] V.P. Feitosa, S. Sauro, T.F. Watson, B. Correr, R. Osorio, M. Toledano, L. Correr-Sobrinho, M.A. Sinhoreti, Evaluation of the micro-mechanical strength of resin bonded–dentin interfaces submitted to short-term degradation strategies, J. Mech. Behav. Biomed. Mater. 15 (2012) 112–120.

[39] S. Sauro, R. Osorio, T.F. Watson, M. Toledano, Assessment of the quality of resin–dentin bonded interfaces: an AFM nano-indentation, μTBS and confocal ultramorphology study, Dent. Mater. 28 (2012) 622–631.
[40] D.H. Pashley, S.M. Thompson, F.P. Stewart, Dentine permeability: effects of the temperature on hydraulic conductance, J. Dent. Res. 62 (1983) 956–959.
[41] J.R. Landis, G.G. Koch, The measurement of observer agreement for categorical data, Biometrics 33 (1977) 159–174.
[42] L.J. Walsh, A.M. Brostek, Minimum intervention dentistry principles and objectives, Aust. Dent. J. 1 (2013) 3–16.
[43] B. Nyvad, M. Kilian, Microbiology of the early colonization of human enamel and root surfaces in vivo, Scand. J. Dent. Res. 95 (1987) 369–380.
[44] A. Kugel, S. Stafslien, B.J. Chisholm, Antimicrobial coatings produced by "tethering" biocides to the coating matrix: a comprehensive review, Prog. Org. Coat. 72 (2011) 222–252.
[45] F. Devinsky, L. Masarova, I. Lacko, D. Mlynarcik, Structure activity relationships of soft quaternary ammonium ampihiles, J. Biopharm. Sci. 2 (1991) 1–10.
[46] R. Liu, Y. Xu, D. Wu, Y. Sun, H. Gao, H. Yuan, Comparative study on the hydrolysis kinetics of substituted ethoxysilanes by liquid-state ^{29}Si NMR, J. Non-Cryst. Solids 343 (2004) 61–70.
[47] Z. Mohammadi, P.V. Abbott, The properties and applications of chlorhexidine in endodontics, Int. Endod. J. 42 (2009) 288–302.
[48] D.L. Scheffel, D.G. Soares, F.G.F.G. Basso, C.A. de Souza Costa, D.H. Pashley, J. Hebling, Transdentinal cytotoxicity of glutaraldehyde on odontoblast-like cells, J. Dent. 43 (2015) 997–1006.
[49] M. Hashimoto, H. Ohno, H. Sano, M. Kaga, H. Oguchi, Degradation patterns of different adhesives and bonding procedures, J. Biomed. Mater. Res. B Appl. Biomater. 15 (2003) 324–330.
[50] J. De Munck, B. Van Meerbeek, Y. Yoshida, S. Inoue, M. Vargas, K. Suzuki, P. Lambrechts, G. Vanherle, Four-year water degradation of total-etch adhesives bonded to dentin, J. Dent. Res. 82 (2003) 136–140.
[51] L. Chen, B.D. Hammond, G. Alex, B.I. Suh, Effect of silane contamination on dentin bond strength, J. Prosthet. Dent. (September) (2016), doi:http://dx.doi.org/10.1016/j.prosdent.2016.06.021.
[52] A.A. Mustafa, J.P. Matinlinna, S. Saidin, M.R. Kadir, The influence of experimental silane primers on dentin bond strength and morphology: a laboratory and finite element analysis study, J. Prosthet. Dent. 112 (2014) 1498–1506.
[53] C.Y.K. Lung, J.P. Matilinna, Resin bonding to silicatized zirconia with two and cross-linking silane, part I: experimental, Silicon 2 (2010) 153–161, doi:http://dx.doi.org/10.1007/s12633-010-9044-9.
[54] C.Y.K. Lung, J.P. Matilinna, Silanes for adhesion promotion and surface modification. Silane, chemistry, applications and performance, in: K. Moriguchi, S.S. Utagawa (Eds.), Silane, Chemistry, Applications and Performance, Nova Science Publishers, New York, 2013, pp. 87–110.
[55] P. Mancheno-Posso, R.F. Dittler, D. Lewis, P. Juang, J.I. XY, X.H. Xu, D.C. Lynch, Review of status, trends, and challenges in working with silane and functional silanes, in: K. Moriguchi, S.S. Utagawa (Eds.), Silane, Chemistry, Applications and Performance, Nova Science Publishers, New York, 2013, pp. 66–87.
[56] K. Hosaka, Y. Nishitani, J. Tagami, M. Yoshiyama, W.W. Brackett, K.A. Agee, F.R. Tay, D.H. Pashley, Durability of resin–dentin bonds to water- vs. ethanol-saturated dentin, J. Dent. Res. 88 (2009) 146–151.
[57] F.T. Sadek, C.S. Castellan, R.R. Braga, S. Mai, L. Tjäderhane, D.H. Pashley, F.R. Tay, One-year stability of resin–dentin bonds created with a hydrophobic ethanol-wet bonding technique, Dent. Mater. 26 (2010) 380–386.
[58] X. Xu, Y. Wang, S. Liao, Z.T. Wen, Y. Fan, Synthesis and characterization of antibacterial dental monomers and composites, J. Biomed. Mater. Res. B Appl. Biomater. 100 (2012) 1151–1162.
[59] Y. Weng, X. Guo, V.J. Chong, L. Howard, R.L. Gregory, D. Xie, Synthesis and evaluation of a novel antibacterial dental resin composite with quaternary ammonium salts, J. Biomed. Sci. Eng. 4 (2011) 147–157.
[60] C.O. Navarra, L. Breschi, G. Turco, M. Diolosà, L. Fontanive, L. Manzoli, R. Di Lenarda, M. Cadenaro, Degree of conversion of two-step etch-and-rinse adhesives: in situ micro-Raman analysis, J. Dent. 40 (2012) 711–717.