Journal of Dentistry xxx (xxxx) xxx–xxx

Contents lists available at ScienceDirect

Journal of Dentistry

journal homepage: www.elsevier.com/locate/jdent

Chlorhexidine-encapsulated mesoporous silica-modified dentin adhesive

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ARTICLE INFO

Keywords:
Chlorhexidine
Mesoporous silica
Adhesive
Dentin
Antibacterial

ABSTRACT

Objectives: This work aims to explore the feasibility of chlorhexidine-encapsulated mesoporous silica (CHX@pMSN) as a modifier of a commercial dental adhesive via the evaluation of physicochemical properties and antibacterial capabilities of adhesive–dentin interface.
Methods: Therapeutic adhesives were developed in the present study by incorporating CHX@pMSN into a commercial adhesive at four mass fractions (0, 1, 5 and 10 wt.%). The antibacterial capability on *Streptococcus mutans (S. mutans)* biofilm, conversion degree, adhesive morphology, microtensile bond strength (MTBS) and nanoleakage expression were evaluated comprehensively.
Results: MTT and CLSM evaluation showed that CHX@pMSN-doped adhesive inhibits *S. mutans* biofilm growth, while CHX is released from the modified adhesive continuously. The incorporation of CHX@pMSN did not affect immediate bond strength at the concentration of 1% and 5% ($P > 0.05$). Moreover, these bonds were mainly preserved in 5% CHX@pMSN group after one month of collagenase ageing. Meanwhile, CHX@pMSN-doped adhesive groups exhibited similar nanoleakage distribution compared with the control.
Conclusion: This study showed that the 5% CHX@pMSN-modified adhesive achieved balance amongst unaffected immediate bonding strength, well-preserved bonds against collagenase ageing and effective inhibition of *S. mutans* biofilm growth.
Clinical significance: CHX@pMSN-modified dentin adhesive can potentially extend the service life of adhesive restoration in clinic.

1. Introduction

As the cornerstone of contemporary aesthetic restoration, dentin bonding systems have already advanced towards the eighth generation or the so-called 'universal adhesives' [1]. This phenomenon drives each manufacturer to rush to acquire this technology, but the attempts appear similar 'putting old wines into new bottles', as stated by Prof. Tay [2]. In other words, poor bonding stability of bonding interface still bothers dentists and entails 60% of practice time to replace failed restoration [3]. This replacement is usually attributed to two main causes: (1) the occurrence of secondary caries at the adhesive–dentin interface due to the accumulation of *Streptococcus mutans (S. mutans)* biofilm [4,5] and (2) the degradation of collagen fibril in the hybrid layer by matrix metalloproteinases (MMPs) and cysteine cathepsins [6,7]. Therefore, dentin adhesives that can inhibit cariogenic biofilms and collagen degradation simultaneously are promising to prolong the durability and service life of adhesive-bonded restoration [8].

Various compositions, such as antibacterial agent, MMP inhibitor and collagen cross-linkers, have been introduced to confer dentin adhesives with multiple functions [8–11]. Amongst these additives, chlorhexidine (CHX), a cationic bis-biguanide, received the most attention [7,12]. Firstly, CHX has been used in dentistry to resist bacterial colonisation and prevent dental plaques due to its wide antibacterial spectrum and nontoxicity towards mammalian cells [13,14]; and secondly, CHX is capable of inhibiting MMPs and cysteine cathepsins, serving as a potent inhibitor of these enzymes to reduce collagen fibril degradation [15–17]. However, simply blending CHX with dental monomer cannot satisfy clinical needs [3] because CHX is immiscible with dental monomers. Thus, CHX is always present in aggregate forms. A porous inferior structure is produced as the dissolution of CHX

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https://doi.org/10.1016/j.jdent.2018.08.012
Received 15 May 2018; Received in revised form 10 August 2018; Accepted 23 August 2018

aggregates in the composite, leading to decreased mechanical properties [18]. Therefore, a reasonable CHX delivery system should be developed to solve this problem.

Mesoporous silica nanoparticles (MSN) is considered as an ideal delivery system for sustained drug release due to its stable framework, tunable pore size, high internal surface area and favourable biocompatibility [19,20]. At present, MSN has been utilised in dental materials as CHX reservoirs to expand the application scope of CHX. For example, Lijian Jin, et al. demonstrated that MSN is capable of encapsulating CHX to fight against oral bacterial biofilms for 24 h [21]; X Xu, et al. reported that CHX-loaded MSN can be applied to modify dental composite with antibacterial capability and continuously release CHX over an extended period [18]; our previous study showed that pore-expanded MSN (pMSN) can carry a high amount of CHX (44.62 wt.%) and that the addition of CHX@pMSN to glass ionomer cement (GIC) resulted in marked anti-biofilm capability without affecting the mechanical performance of GIC [22]. However, to the best of our knowledge, no reports on dentin adhesive modification with CHX@pMSN are available. We speculate that CHX@pMSN may find its stage in dentin bonding due to the slow-release characteristic of pMSN and the dual role (antibacterial agent and MMP inhibitor) of CHX itself.

The aim of this study is to explore the feasibility of CHX@pMSN as a versatile modifier in dentin bonding via the evaluation of physicochemical properties and antibacterial capability of the adhesive–dentin interface. The following are the null hypotheses: CHX@pMSN-incorporated dentin adhesive (1) does not kill *S. mutans* or inhibit biofilm growth; and (2) does not affect bonding properties even after the occurrence of collagenase ageing.

2. Materials and methods

All chemical reagents, including cetyltrimethyl ammonium bromide (CTAB) (Mw 364.45), tetraethyl orthosilicate (TEOS), mesitylene (TMB), CHX diacetate salt hydrate and *Clostridium histolyticum* collagenase, were purchased from Sigma (St. Louis, MO, USA). All chemicals were used as received.

2.1. Preparation of CHX@pMSN

Prior to CHX loading, pMSN was synthesised on the basis of a previously reported technique with several modifications [23]. In brief, 1 g of CTAB was dissolved in a solution containing 480 mL water and 3.5 mL of 2 N NaOH; then, 7 mL of TMB was incorporated as a pore swelling agent. After being stirred vigorously at 80 °C for 4 h, the solution was added with 5 mL of TEOS, stirred for 2 h and then centrifuged, washed and oven-dried at 60 °C overnight. After calcining in air at 550 °C for 5 h, the pMSN was obtained. A total of 50 mg of CHX was dissolved in 5 mL of pure ethanol, incorporated with 50 mg of pMSN, sonicated for 10 min and gently shaken for 24 h at room temperature. The mixture was centrifuged, briefly rinsed with ethanol and vacuum dried before use. The morphology characteristics of pMSN and CHX@pMSN were observed using TEM (JEM-1230, JEOL, Tokyo, Japan).

2.2. Preparation of experimental adhesives

A commercially available dentin adhesive called Adper Scotchbond Multipurpose (SBMP) (3 M ESPE, St. Paul, MN, USA) was used as the parent material. Then, three distinct CHX@pMSN amounts (1, 5 and 10 wt.%) were incorporated into the adhesive resin (w/v) of the SBMP system. SBMP served as the control without the addition of CHX@pMSN.

2.3. Preparation of specimens for antibacterial test

The cover of a sterile 96-well plate was used as a mould for specimen preparation. A total of 30 μL of experimental adhesives was coated on the bottom of a sterile 96-well cover (eight samples for each group), followed by curing with a light unit (Bluephase Style, Ivoclar-Vivadent Amherst, NY, USA) for 20 s. The cured specimen was approximately 6.4 mm in diameter and 1 mm in thickness. For the ageing process, four prepared specimens from each group were stored in distilled water that was renewed every 3 days. After 30 days at 37 °C, specimens were subjected to the following antibacterial test.

S. mutans UA159 was provided by the School of Stomatology, Wuhan University. The bacteria were incubated at 37 °C for 24 h in brain heart infusion broth anaerobically, and then the suspension was adjusted to 1×10^8 CFU/mL for further usage. After being dried and disinfected, all specimens were transferred into the wells of a 24-well plate. Then, 10 μL of *S. mutans* cell suspension (10^8 CFU/mL) was transferred to each well, which contained 1 mL of BHI with 1% sucrose, and anaerobically incubated at 37 °C for 24 h. The non-adherent bacteria cells of biofilm-coated specimens were gently washed away by 1 mL of sterile phosphate buffer solution (PBS).

2.4. Antibacterial evaluation by MTT assay

3-(4,5-Dimethylthiazol-2-yl)-2,5-diphenyltetrazolium bromide kit (MTT; Sigma Chemical Co., St. Louis, USA) was used to prepare 0.5 mg/mL MTT solution. Two biofilm-coated specimens from each group were transferred to a 12-well plate, followed by incorporation with 1 mL of MTT solution. After anaerobic incubation at 37 °C for 1 h, the MTT solution was pipetted out and replenished with 1 mL of dimethyl sulfoxide (DMSO). The plate was gently shaken for 20 min, and the absorbance of the supernatant at 570 nm was measured using a spectrophotometer (Powerwave 340, Bio-tek Instruments, Winooski, VT, USA).

2.5. Confocal laser scanning microscopy analysis

S. mutans biofilms adhering on the specimen with one-month water storage were analysed through confocal laser scanning microscopy (CLSM) analysis (Fluoview FV1200, Olympus, Tokyo, Japan). Specifically, two biofilm-coated specimens from each group were stained by the Live/Dead Bacterial Viability Kit (Molecular Probes, Invitrogen, USA) for 15 min. The specimens were rinsed gently and observed via CLSM at 40× magnification. Excitation at 488 nm wavelength demonstrated the green fluorescence of live bacteria stained by SYTO-9, whereas excitation at 543 nm wavelength demonstrated the red fluorescence of dead bacteria stained by propidium iodide (PI). Continuous scanning along the Z-stack of 20 μm produced 10 images from the adhesive surface to the top of the biofilm. 3D overlay images along the Z-stack was reconstituted by Imaris 7.2.3 software (Bitplane, Zürich, Switzerland), whilst the distributions of live/dead bacterial at each layer were plotted.

2.6. CHX release

Three adhesive disks (Ø 8 mm, H 1 mm) for each group were prepared with the aid of a polytetrafluoroethylene mould with circular holes followed by curing with a light unit (Bluephase Style, Ivoclar-Vivadent Amherst, NY, USA) for 20 s. Each sample was immersed in 5 mL of sterile deionised water at 37 °C. The CHX released from the experiment adhesives were measured at 1, 3, 7, 10 and 30 days. At each time point, 1 mL of the solution was removed and replaced with fresh distilled water. The removed aliquots were analysed for CHX content by high-performance liquid chromatography (Agilent1100, Palo Alto, CA, USA). The cumulative release profile of CHX from each experiment adhesive groups was plotted.

2.7. Degree of conversion (DC)

The DC of the experimental adhesives was analysed by real-time