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ORIGINAL ARTICLE

EDTA-chitosan is a feasible conditioning agent for dentin bonding

Yi Zhou[1] · Yuming Zhao[1] · Jianmin Han[2,3]

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Abstract

Objectives The bonding effects of EDTA-chitosan, phosphoric acid, and SE-Bond were compared.

Materials and methods Material synthesis, Fourier transform infrared spectroscopy, transmission electron microscopy, scanning electron microscopy, laser confocal microscopy, microtensile bond strength, stereomicroscope observation section, CCK8 cytotoxicity assay, and microfluidic experiments were applied.

Results EDTA-chitosan was synthesized, and it was found by transmission electron microscopy that the application of EDTA-chitosan to dentin can extrafibrillarly demineralize collagen fibers. Scanning electron microscopy provided evidence for the retention of smear plugs in dentin conditioned with 1 wt% EDTA-chitosan. Mixed layer and long resin protrusions can be formed after bonding under a laser confocal microscope. The microtensile strength test found that the bonding strength and the durability obtained by applying the chelating agent EDTA-chitosan to dentin were equivalent to SE-Bond and better than the phosphoric acid wet bonding commonly used clinically ($P < 0.05$). The cytotoxicity of EDTA-chitosan was lower than that of phosphoric acid and SE-Bond in the CCK-8 assay and lower than that of phosphoric acid in the microfluidics experiment.

Conclusions Taken together, the EDTA-chitosan extrafibrillar demineralization strategy retains intrafibrillar minerals and provides better bonding strength and durability with lower cytotoxicity.

Clinical relevance EDTA-chitosan has the potential to be applied to dentin resin for direct bonding restoration and has good clinical application prospects.

Keywords Chelation · Dentin bonding · Collagen fibers · Durability

Introduction

Resin direct bonding repair is the most common treatment for oral cavity repair due to its good aesthetics and simple operation by the side of the chair. The bonding mainly involves bonding to enamel and dentin. The bonding to enamel is relatively reliable, but the bonding effect to dentin is not ideal due to its special structure, including a large number of high-permeability dentin tubules that cause problems during wet bonding. Therefore, dentin bonding has always been challenging and is a research hotspot in the field of oral bonding.

To improve the durability of dentin bonding, many methods have been explored. Recently, extrafibrillar dentin demineralization was proposed as a new conceptual bonding strategy for enhancing resin-dentin bond stability [1]. This concept is based on the size exclusion principle, in which molecules > 40 kDa are completely excluded from the intrafibrillar water compartments of fibrillar collagen [2]. High molecular weight polymeric chelating agents can selectively remove apatite crystallites from the extrafibrillar spaces of the mineralized dentin matrix. Unlike the action of small molecular weight acids and monomeric acidic resin monomers that progressively dissolve extra- and intrafibrillar minerals, intrafibrillar minerals are preserved to retain the mechanical strength of collagen fibers, which enables

Yuming Zhao and Jianmin Han are co-equal senior authors

✉ Yuming Zhao
yuming_zhao@hotmail.com

✉ Jianmin Han
hanjianmin@bjmu.edu.cn

[1] Department of Pediatric Dentistry, School and Hospital of Stomatology, Peking University, No. 22 South Street, Zhongguancun, Haidian District, Beijing 100000, China

[2] Department of Dental Materials, School and Hospital of Stomatology, Peking University, No. 22 South Street, Zhongguancun, Haidian District, Beijing 100000, China

[3] Dental Medical Devices Testing Center, Peking University School and Hospital of Stomatology, Beijing, China

air-drying of the partially demineralized dentin to eliminate problems associated with water-wet bonding in a clinical setting [1]. Theoretically, any biocompatible chelating agent can be used for dentin demineralization, and different molecular sizes and action times will affect the demineralization efficiency. Some researchers have used high molecular weight polyacrylic acid salts and chitosan as calcium-chelating agents [1, 3].

Chitosan is a proteoglycan derived from a wide range of sources in nature, and it is easily obtained, consisting of β-(1–4)-linked 2-acetamido-2-deoxy-β-D-glucopyranose and 2-amino-2-deoxy-β-D-glycopyranose. Chitosan is biocompatible, biodegradable, environmentally friendly, antibacterial, and minimally immunogenic. Hence, chitosan and its derivatives play an important role in many fields, such as the medical field, food preservation, water treatment, cosmetics, and agriculture [4–7]. As a chelating agent, chitosan was found to demineralize dentin extrafibrillarly when used at low concentrations. Meanwhile, there was no difference in the bond strengths of experimental adhesives applied with dry bonding on dentin conditioned with high molecular weight chitosan for 60 s or wet bonding with phosphoric acid for 15 s [3]. However, the operation duration was unsatisfactory, so new chelating agents should be developed with a higher chelating efficiency, and their durability should be tested.

This study considered that the introduction of the carboxyl group of EDTA into chitosan could enhance its chelating ability [8], so the 1-ethyl-3-(3-dimethylaminopropyl) carbodiimide (EDAC) chemical cross-linking agent was used to cross-link EDTA and chitosan to make part of the amino group of chitosan combined with part of the carboxyl group of EDTA to synthesize EDTA-chitosan. Ethylenediamine tetraacetic acid (EDTA) has a strong calcium-chelating ability with four carboxylic acid groups [9]. Currently, it is widely used as an endodontic irrigant during root canal preparation [10–14]. To investigate the potential of using high molecular weight EDTA-chitosan to demineralize and bond dentin, it was compared with phosphoric acid and SE-Bond, which are clinically used. Additionally, a chitosan control group was set to determine whether the chelating efficiency had been improved by the chemical modification.

Materials and methods

Specimen collection and preliminary processing

Noncarious human third molars were collected according to a protocol approved by the Research Ethical Committee. The teeth were stored at 4 °C in chloramine-T to control bacterial growth and transferred to distilled water after a week. All teeth were used within 6 months. A water-cooled low-speed diamond saw was used to create a flat surface of midcoronal dentin perpendicular to the longitudinal axis. Teeth with exposed pulp were excluded. A smear layer was produced by wet sanding the bonding surface with 600-grit silicon carbide paper for 1 min under water irrigation. All experimental procedures were performed by the same trained operator.

The teeth were randomly divided into 7 groups, in which the dentin surfaces were treated with water, 35% phosphoric acid (Heraeus, Germany) for 15 s, SE primer for 20 s (Clearfil, Japan), 2 wt% EDTA for 60 s, 1% (v/v) acetic acid for 60 s, 1 wt% chitosan for 60 s, or 1 wt% EDTA-chitosan for 60 s. The liquid conditioners were liberally applied to the dentin surface and agitated with a microbrush. SE primer was applied for 20 s, and then the surface was dried with mild air flow with no rinsing, while in other groups, rinsing was necessary. The 7 groups were named the blank control, PA, SE, EDTA1, AA1, C1, and EDTAC1 groups.

Material synthesis

EDTA-chitosan was synthesized as previously reported [8]. Carbodiimide hydrochloride (1-ethyl-3-(3-dimethyl aminopropyl) carbodiimide hydrochloride, EDAC·HCl) (Shyuanye, China) was used to chemically cross-link EDTA and chitosan to form EDTA-chitosan. First, chitosan (50 kDa, Macklin, China) was dissolved in 1% (v/v) acetic acid, and EDTA-2Na (the mass ratio of EDTA to chitosan was 2:1) was added to the solution. Then, the pH was adjusted to 5.0 and EDAC·HCl was added. The mixture was stirred at room temperature for 4 h and then dialyzed and freeze-dried to prepare the finished product. It was stored at 4 °C until further use.

A 1 wt% EDTA-chitosan solution was prepared by dissolving the composite in 1% (v/v) acetic acid. The pH value was measured with a pH meter (FiveEasy Plus, Mettler Toledo, Switzerland).

Structural identification

To evaluate the synthesis, the structure of the composition and reactants was observed by Fourier transform infrared spectroscopy (Nicolet iS50 FT-IR, Thermo Fisher Scientific, America): 100-mg KBr was mixed with 1-mg sample to prepare the samples. Then, the samples were scanned in the frequency range of 4000–400 cm^{-1}, and data were collected at a resolution of 4 cm^{-1}.

Transmission electron microscope

To validate the extrafibrillar demineralization concept, 7 groups of segments were prepared ($n = 3$). Then, after rinsing with deionized water, the conditioned crown segments were fixed in Karnovsky's fixative and postfixed in

1% osmium tetroxide. Each specimen was dehydrated in an ascending ethanol series (50 to 100%), immersed in propylene oxide, and embedded in epoxy resin. Nondemineralized 70-nm-thick sections were examined unstained with a JEM-1400 transmission electron microscope (JEOL, Japan) at 110 keV.

Scanning electron microscope

To observe the smear layer after treatment with chelating agents or etching agents, 7 groups of segments were prepared ($n = 2$), and the specimens were air-dried and kept in a desiccator containing anhydrous calcium sulfate for 24 h. The specimens were secured, sputtered with gold/palladium, and examined using a conventional high-vacuum SEM (JSM-7900F, JEOL, Japan) operating at 10–15 kV.

Laser confocal microscope

The mixed layer and resin protrusion formed after bonding was observed with a laser confocal microscope. Rhodamine B was dissolved in SE-Bond and SL-Bond adhesives. The 7 groups of segments were prepared ($n = 3$), and then the adhesive was applied. The microscopic morphology of the longitudinal section of the resin-dentin surface was observed with a laser confocal microscope (TCS SP8, Leica, Germany), the excitation wavelength of which was 561 nm.

Microtensile bond strength

Immediate bond testing (n = 18)

PA: Phosphoric acid was used to etch the surface of the dentin for 15 s, and then the dentin was rinsed thoroughly with distilled water for at least 20 s. We blew off the excess water with a gentle air flow and kept the dentin moist. Then, we applied SL bond (Swiss, Coltene) and left it for 20 s under a gentle air flow. The adhesive was light cured for 10 s (according to the instructions).

SE: The primer was applied for 20 s and the surface was dried under gentle air flow. Then, the bond was applied and left for 20 s under gentle air flow. The adhesive was light cured for 10 s (according to the instructions).

EDTA1, AA1, C1, EDTAC1: The chelating agent was applied for 60 s, and then the dentin was rinsed thoroughly with distilled water for at least 60 s. We dried the surface with a gentle air flow, applied the SL bond, and left it for 20 s under a gentle air flow. The adhesive was light cured for 10 s.

After light curing of each adhesive, two 2-mm-thick layers of a light-curable resin composite (3 M ESPE FiltekTM Z350 XT, America) were placed over the bonded dentin. Each layer was polymerized for 10 s. All specimens used for bond strength evaluation were stored in deionized water at 37 °C for 24 h prior to sectioning.

Then, we used a low-speed cutting machine to cut perpendicular to the bonding surface to obtain a test piece with a cross section of approximately 1 mm × 1 mm so that the bonding area of each test piece was approximately 1 mm^2. The actual bonding area of each test piece was measured and calculated with a micrometer.

An Ergo 5400 was used to fix the bonding test piece on the universal testing machine. We stretched the test piece at a loading speed of 0.5 mm/min and tested the maximum load force (N) when it broke perpendicular to the bonding surface and calculated the microtensile strength (MPa) = breaking load (N)/bonding area (mm^2).

Aging under 5000 hydrothermal cycles (n = 18)

All samples in EDTA1 group and AA1 group suffered pretest failures during the cutting of specimens. Hence, the other 4 groups were selected to participate in the aging experiment. After curing the resin, all groups were challenged with hydrothermal cycles (5000 hydrothermal cycles at 5 and 55 °C) before bond testing, corresponding to 0.5 years of intraoral use [15]. The other procedures were the same as those for immediate bond testing.

Stereomicroscope observation section

The interface of the resin and dentin was observed after bond testing under a stereomicroscope (SMZ 1500, Nikon, Japan), and the frequency of failure mode was recorded ($n = 18$). Failure modes were classified as adhesive (within the adhesive), interface (between the dentin and adhesive), cohesive (within the dentin or within the composite buildup), or mixed failure (both adhesive and cohesive).

CCK8 cytotoxicity assay

Cell viability was monitored with a CCK-8 kit (CCK-8, Dojindo, Japan) following the manufacturer's instructions. The fifth generation of human dental pulp stem cells (hDPSCs) in logarithmic growth phase was cultivated in 96-well plates. Then, 35% phosphoric acid, SE-Bond, 2 wt% EDTA, 1% (v/v) acetic acid, 1 wt% chitosan, or 1 wt% EDTA-chitosan was diluted 100 times in 10% FBS α-MEM medium, which was set as the control ($n = 3$). The cells were incubated for 24 h and observed under an inverted light microscope (CKX41, Olympus, Japan). Then, CCK8 solution was added to each well and incubated for 1 h, and the absorbance (optical density, OD value) was detected with a microplate reader (Elx808, Biotek, America) at a wavelength of 450 nm. The relative growth rate (RGR) of each group was calculated as

RGR = (experimental group − blank control group)/(negative control group − blank control group) × 100%.

Microfluidic experiments

To simulate the real structure of the dental pulp-dentin complex and establish a "material-dentin-dental cell" model, a microfluidic model was designed and used for EDTA-chitosan material cytotoxicity testing. A microfluidic chip was designed as shown in Fig. 1, which included a 4-mm-diameter dental pulp cell chamber and a 1-mm groove for the flow of medium fluid. Polydimethylsiloxane (PDMS) was used to make the chips. A 0.5-mm-thick dentin chip was placed under the dental pulp cell chamber and firmly fixed on the PDMS by a pressure plate. The other side of the dentin exposed a surface to be used for applying the dental materials. The chip, water pump, and catheter together formed a microfluidic device. Before the experiment, the dentin flakes were treated with 17% EDTA for 45 s to promote cell adhesion and then stored in distilled water. All supplies were sterilized by ultraviolet light before use.

hDPSCs (20 µl 10^5/ml) were inoculated into the cell chamber of the microfluidic chip, and then the chamber was filled with the cell growth medium. The circulation pipe started to circulate the culture medium at a rate of 2 ml/h. The cells were incubated to promote contact and attachment to the dentin surface. After 24 h, dental materials were applied to the other side of the dentin chip. The 7 groups were treated with water, 35% phosphoric acid for 15 s, SE-Bond for 20 s, 2 wt% EDTA for 60 s, 1% (v/v) acetic acid for 60 s, 1 wt% chitosan for 60 s, and 1 wt% EDTA-chitosan for 60 s ($n = 2$). Finally, 2-µM calcein-AM/4.5-µM PI (Solarbio, China) was used to stain the live and dead cells, and the stained cells were observed with a laser confocal microscope under 561-nm excitation light.

Statistical analyses

All experimental data were analyzed with SPSS 25.0 (IBM) with significance preset at $\alpha = 0.05$. Owing to the heteroscedastic nature of the datasets, nonparametric statistical analyses were performed. Analysis was performed with 1-factor analysis of variance (ANOVA) and Dunnett's T3 statistic to examine whether a significant difference existed in the groups. Fisher's exact test was used to compare the distribution of the failure modes.

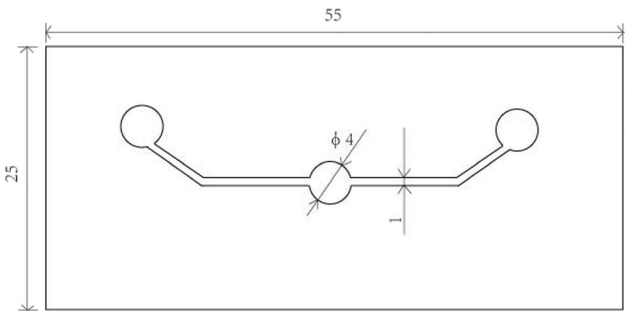

Fig. 1 Microfluidic chip

Results

The synthesis conditions referred to previous studies, and the product was subjected to Fourier transform infrared spectroscopy after synthesis, which proved that EDTA and chitosan had undergone an acylation reaction [12].

EDTA-chitosan was synthesized and dissolved in 1% (v/v) acetic acid solution. The 1 wt% EDTA-chitosan liquid appeared brownish yellow translucent (pH 3.36).

Material structure characterization

Figure 2 shows Fourier transform infrared spectroscopy images of EDTA, chitosan, and EDTA-chitosan. Compared with chitosan and EDTA, the peak of EDTA-chitosan at 3300 ~ 3500 cm^{-1} was significantly weakened, which proved that the content of $-COOH$ and $-NH_2$ in the product was significantly reduced. Meanwhile, the new characteristic peak near 1600 cm^{-1} proved the existence of CO–NH, which confirmed the successful synthesis of EDTA-chitosan.

Dentin surface microstructure

Figure 3 shows transmission electron microscopy images of dentin surfaces conditioned with different chelating agents or etching agents. EDTA-chitosan was found to create a 500-nm-thick partially demineralized zone (between the yellow arrows) on the dentin surface, and collagen fibrils with intrafibrillar apatite crystallites could be identified within the partially demineralized zone. The partially demineralized zone was 250 nm thick after 60 s of chitosan conditioning, and intrafibrillar apatite was still retained within the collagen fibrils. While the phosphoric acid created a 5–7-µm completely demineralized zone, collagen fibrils could not be identified. Similarly, EDTA created a 1–2-µm completely demineralized zone. In the SE-Bond group, a 500-nm-thick demineralized zone was created, the surface layer was completely demineralized, and the deep layer was partially demineralized. Application of the acetic acid solvent for 60 s did not result in surface demineralization.

Scanning electron microscopy images of dentin are shown in Fig. 4. In the air-dried phosphoric acid specimens, removal of water from the interfibrillar spaces resulted in collapse of the completely demineralized collagen network. Nearly all of the smear plugs in the dentinal tubules and the

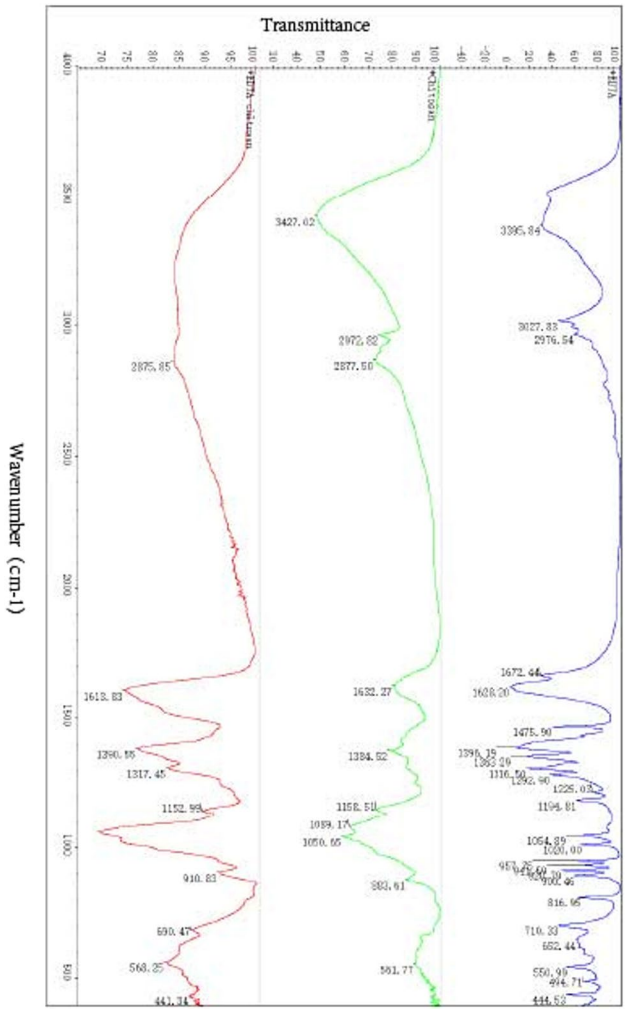

Fig. 2 Fourier transform infrared spectroscopy of EDTA, chitosan, and EDTA-chitosan. The blue line was EDTA. The green line was chitosan. The red line was EDTA-chitosan

smear layer were completely removed after rinsing. SE-Bond mostly removed the smear layer and left part of the smear plugs. The EDTA and acetic acid specimens did not seem to change compared with the blank control in **a**. Specimens conditioned with chitosan remained mostly as smear layers and smear plugs. EDTA-chitosan removed most of the smear layer and smear plugs, the chelating efficiency of which was weaker than that of phosphoric acid and SE-Bond but higher than that of EDTA, acetic acid, and chitosan.

Figure 5 shows laser confocal microscope images of dentin surfaces. The length of the resin protrusions of phosphoric acid was the longest, up to 200 μm, followed by EDTA-chitosan, SE-Bond, and chitosan, which were approximately 100 μm. The resin protrusions formed by EDTA and acetic acid were extremely short and thin, less than 50 μm.

The thickness of the mixed layer of SE was the thickest, followed by phosphoric acid, EDTA-chitosan, and chitosan, while the EDTA and acetic acid could only form a mixed layer.

Dentin bonding

The microtensile bond strength evaluation is summarized in Fig. 6. The EDTA and the acetic acid groups failed before bond testing. The comparisons indicated that the bond strengths of EDTA-chitosan to air-dried dentin were significantly higher than that of 35% phosphoric acid to wet dentin ($P < 0.05$), and there was no significant difference between EDTA-chitosan and SE-Bond ($P > 0.05$). After 5000 hydrothermal cycles, the difference between EDTA-chitosan and phosphoric acid was more significant ($P < 0.01$). Additionally, there was no significant difference between EDTA-chitosan and SE-Bond ($P > 0.05$). In addition, the bond strengths of EDTA-chitosan were significantly higher than those of chitosan ($P < 0.05$).

The results of the failure mode tests showed that mixed failure was the most frequent mode in all groups in Fig. 7. Adhesive failure accounted for a small number. No cohesive failures were seen. EDTA and the acetic acid groups all failed at the interface. There was no significant difference among the other groups.

Cytotoxicity

Dental materials and cells were cocultivated in direct contact. Figure 8 shows that the RGR of EDTA-chitosan was grade 0, and the RGR of phosphoric acid and the SE bonds was grade 4. Cells in the EDTA-chitosan, chitosan, EDTA, and acetic acid groups had good cell morphology and a spindle shape. In contrast, almost all cells in the phosphoric acid and SE-Bond groups died (Fig. 9).

Microfluidic experiments showed that the cells of all groups attached to dentin to form a single layer, and there was no cell apoptosis. Cells in the phosphoric acid group were more rounded than that in the other groups, in which the cells exhibited a typical spindle-like morphology (Fig. 10).

Discussion

The concept of extrafibrillar dentin demineralization was proven in this study. As shown in the results, EDTA-chitosan conditioning created a partially demineralized zone with collagen fibrils containing intrafibrillar apatite crystallites. Meanwhile, the demineralized zone was deeper than with chitosan, which proved that EDTA-chitosan was more efficient. Our chitosan results are consistent with previous studies [3]. Surprisingly, we found that EDTA-chitosan had a deeper demineralization than chitosan, which may be due

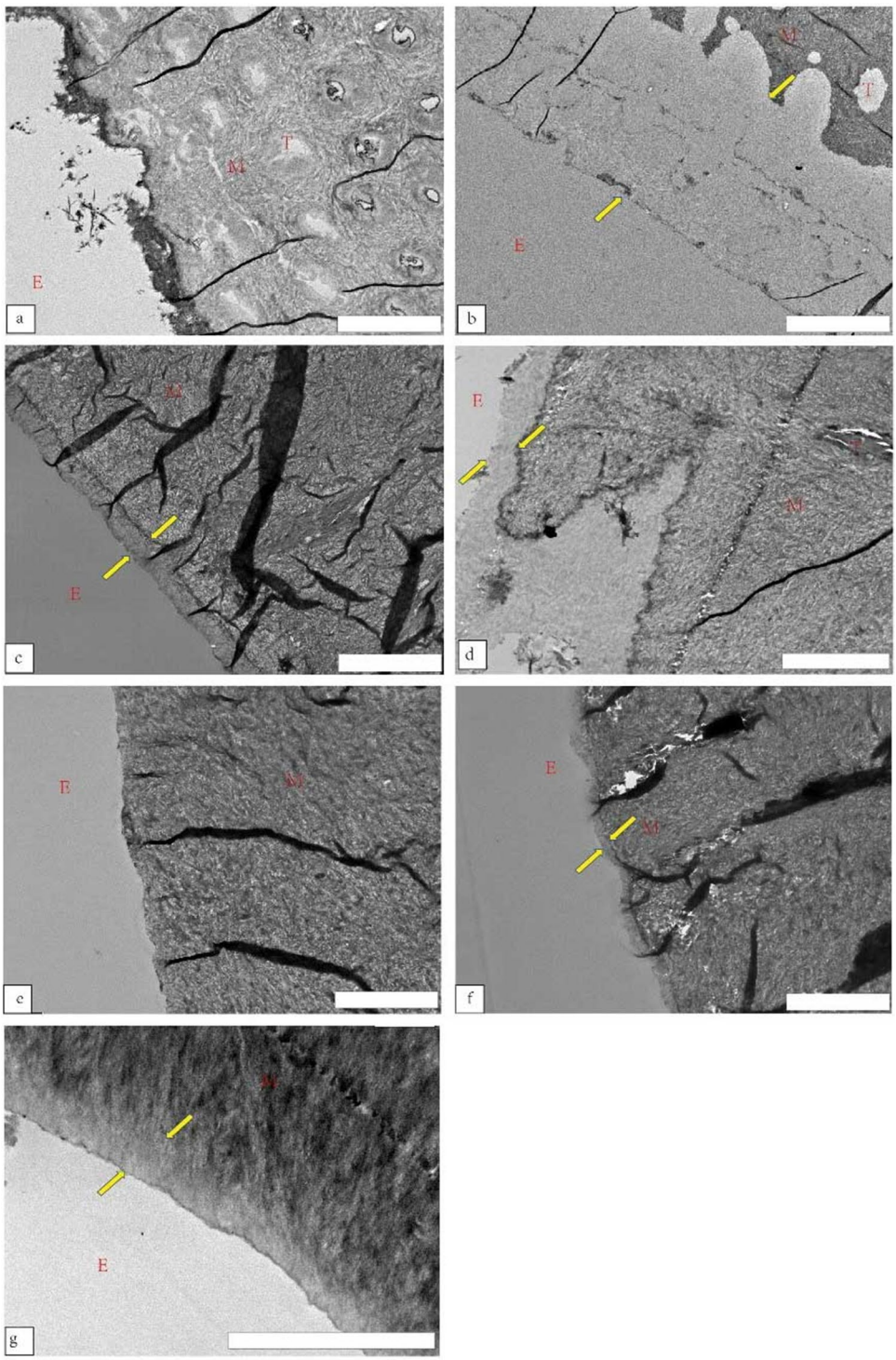

Fig. 3 **a**, **b**, **c**, **d**, **e**, **f**, **g** Transmission electron microscopy of unstained nondemineralized dentin after the surfaces were conditioned with water, 35% phosphoric acid for 15 s, SE-Bond for 20 s, 2 wt% EDTA, 1% (v/v) acetic acid, 1wt% chitosan, or 1 wt% EDTA-chitosan for 60 s (bar: 5 μm). E, epoxy resin; M, mineralized dentin; T, dentinal tubule. Yellow arrows showed the demineralized zone

to the introduction of carboxyl groups into the macromolecular chitosan to enhance its ability to capture calcium ions. In addition, the demineralization of acetic acid was insignificant. Thus, the extrafibrillar dentin demineralization by EDTA-chitosan was attributed exclusively to its chelating capacity and not the efficiency of the solvent. Benefitting from intrafibrillar minerals that were not removed, the partially demineralized dentin matrix did not collapse when air-dried. Therefore, the air-dry bonding method was used in the subsequent microtensile bond strength experiment.

This study provided evidence for retention of smear plugs in dentin conditioned with EDTA-chitosan, and mixed layer and long resin protrusions could be formed. The bonding strength of EDTA-chitosan was stronger than that of

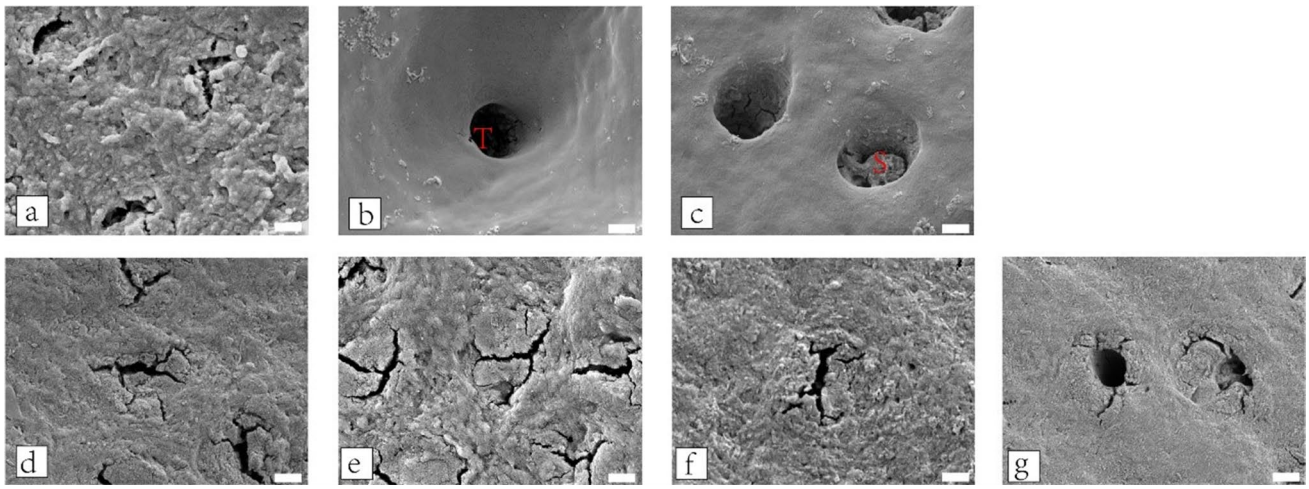

Fig. 4 **a**, **b**, **c**, **d**, **e**, **f**, **g** Scanning electron microscopy of dentin after the surfaces were conditioned with water, 35% phosphoric acid for 15 s, SE-Bond for 20 s, 2 wt% EDTA, 1% (v/v) acetic acid, 1wt% chitosan, or 1 wt% EDTA-chitosan for 60 s (bar: 1 μm). T, dentinal tubule; S, smear plugs

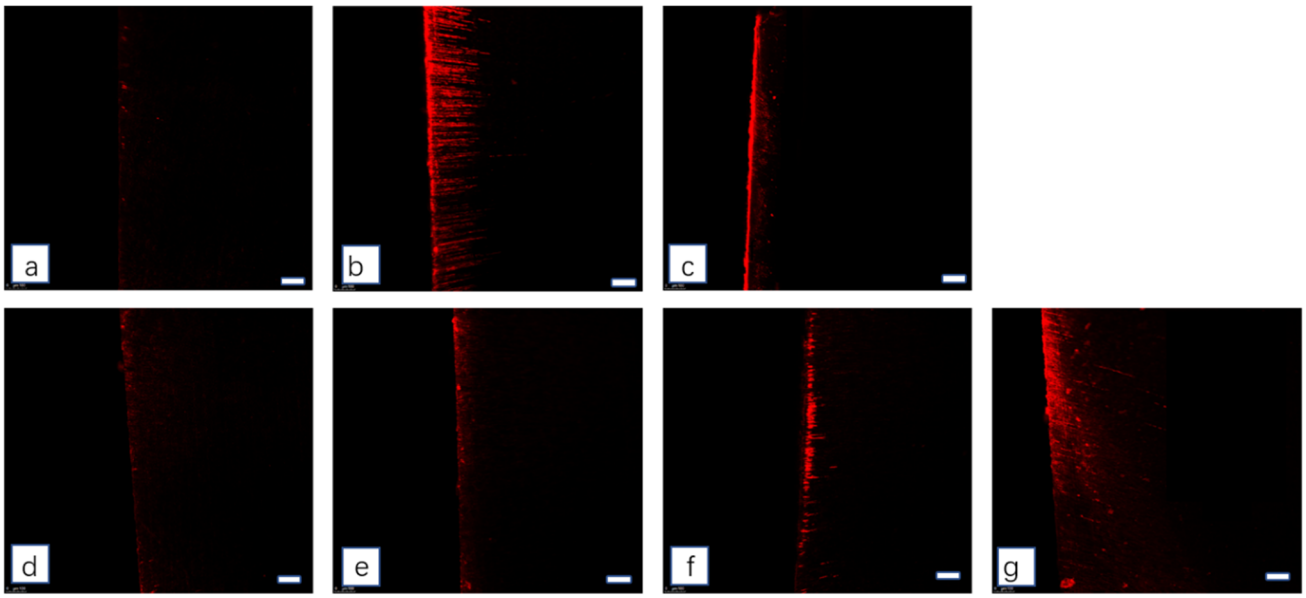

Fig. 5 **a**, **b**, **c**, **d**, **e**, **f**, **g** Laser confocal microscope images of dentin after the surfaces were conditioned with water, 35% phosphoric acid for 15 s, SE-Bond for 20 s, 2 wt% EDTA, 1% (v/v) acetic acid, 1wt% chitosan, or 1 wt% EDTA-chitosan for 60 s (bar: 100 μm)

phosphoric acid wet bonding, which was equivalent to that of chitosan and SE-Bond, and the durability of EDTA-chitosan was the best. The etch-and-rinse technique is generally not compatible with dry bonding because the collapse of the completely demineralized dentin matrix impedes resin infiltration [16, 17]. When water is incompletely replaced during adhesive infiltration, the residual water provides a medium for hydrolysis of the polymerized resin network and proteolytic destruction of the denuded collagen [18–21]. Elimination of these factors via EDTA-chitosan chelation contributed to the stability of the resin-dentin interface, as demonstrated by the 5000-hydrothermal cycle bonding test. The use of an additional rinsing step may be cumbersome compared with the use of self-etching. However, it could eliminate the problems associated with the use of hydrophilic self-etch and universal adhesive formulations.

The bond strength results showed that there was no significant difference between SE and EDTA-chitosan ($P > 0.05$). Compared with the use of self-etching primers/adhesives, intrafibrillar minerals were not removed in the

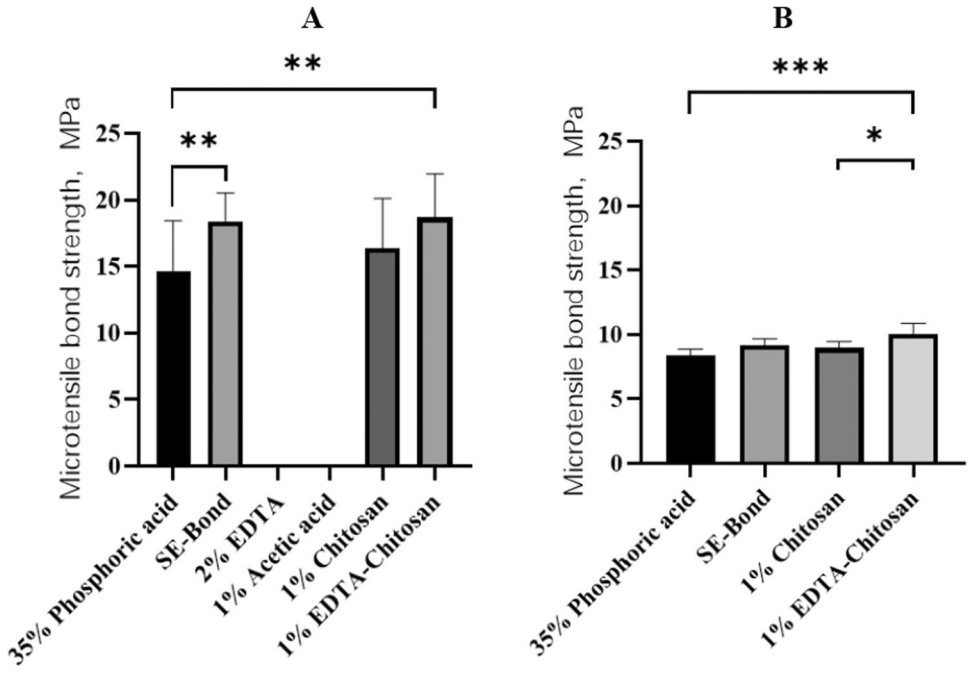

Fig. 6 Microtensile bond strength of **A** immediate and **B** 5000 times of hydrothermal cycles. *$p < 0.05$, **$p < 0.01$, ***$p < 0.001$

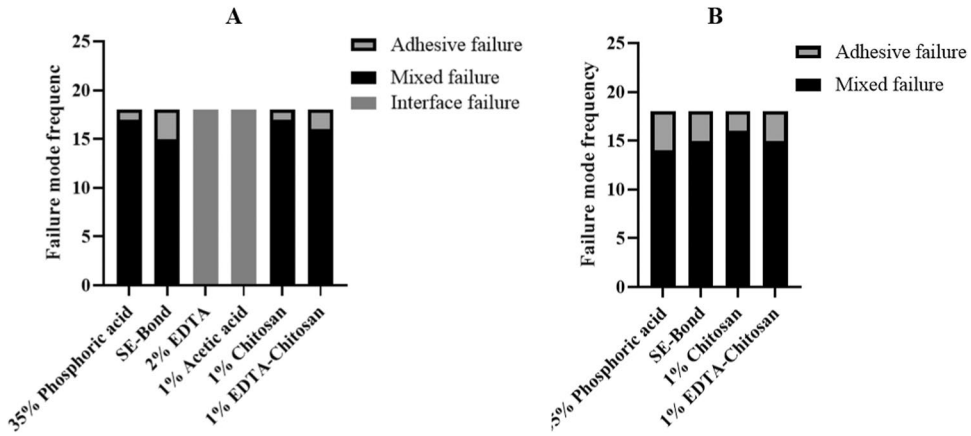

Fig. 7 Failure modes of different groups, **A** immediate; **B** 5000 times of hydrothermal cycles

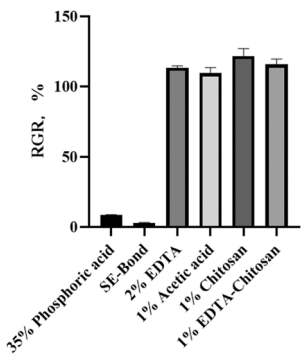

Fig. 8 CCK8 assay results showed the RGR of different groups

EDTA-chitosan group, as TEM showed. The partially demineralized dentin matrix allowed air-drying, which provided the advantage of less hydrophilic adhesive resin monomers for bonding and less water absorption and dissolution after polymerization [22]. Therefore, a higher tensile strength of immediate and hydrothermal cycle bond testing in the EDTA-chitosan group was expected. The actual results could be due to a smear layer remaining, as SEM showed, which blocked the combination of dentin and adhesive. Therefore, we hope to improve the bonding effect by improving the performance of the material and removing the smear layers in the later stages.

It has been found that many etching agents and resin after curing have cytotoxic effects and even affect the endocrine system of the body [23, 24]. Therefore, the detection of cytotoxicity of dental materials is necessary. By cocultivating the materials and pulp cells directly, the reaction of the cells to the material could be observed most intuitively. The RGR of EDTA-chitosan was grade 0, and the cytotoxicity was obviously better than that of phosphoric acid and SE-Bond,

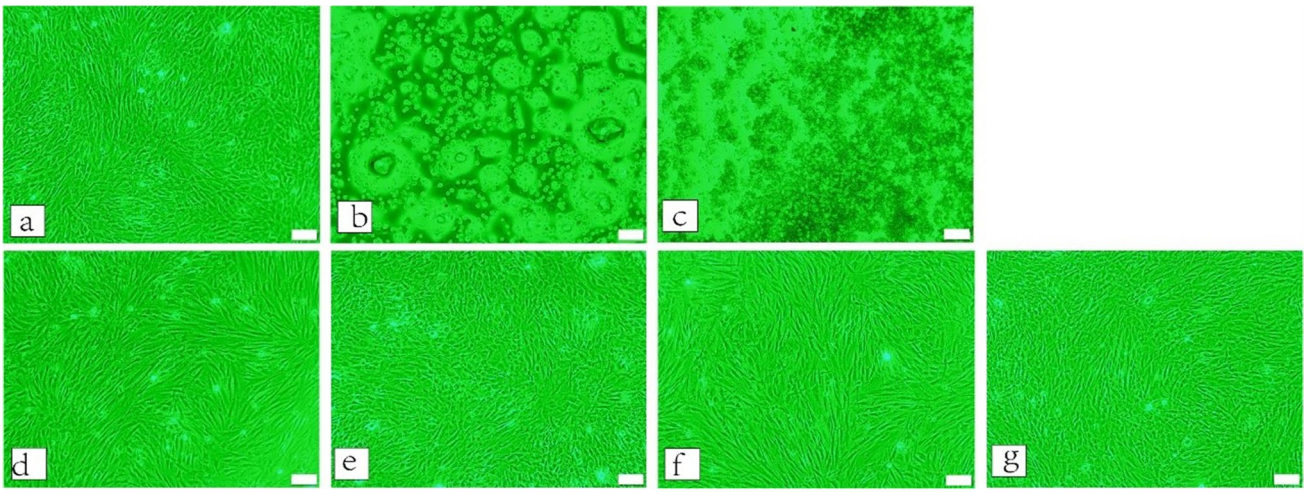

Fig. 9 Cells were observed under inverted light microscope. **a**, **b**, **c**, **d**, **e**, **f**, **g** Inverted light microscope images of blank control, phosphoric acid, SE-Bond, EDTA, acetic acid, chitosan, or EDTA-chitosan (bar: 100 μm)

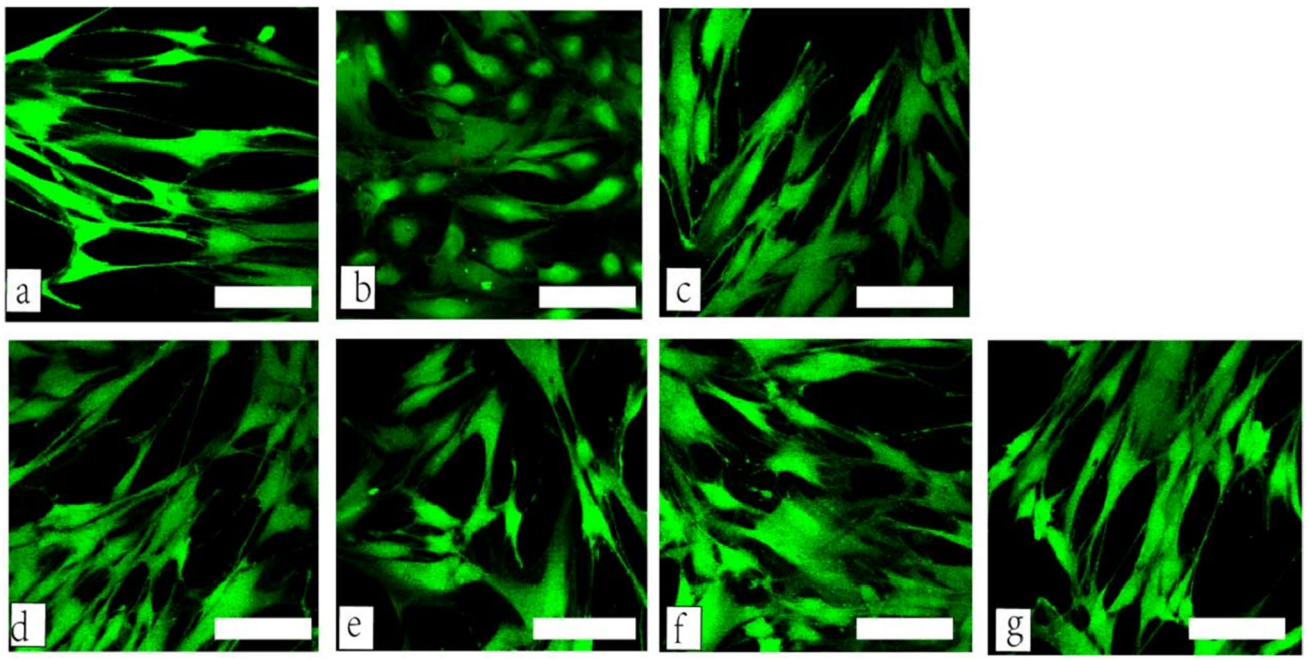

Fig. 10 Stained cells in microfluidic experiments were observed under laser confocal microscope. **a**, **b**, **c**, **d**, **e**, **f**, **g** Laser confocal microscope images of blank control, phosphoric acid, SE-Bond, EDTA, acetic acid, chitosan, or EDTA-chitosan (bar: 100 μm). Dead cells were dyed red, which could not be seen in all groups. Live cells were dyed green

proving that the chemically cross-linked EDTA-chitosan retained the good biocompatibility of chitosan [25]. The excellent biocompatibility of EDTA-chitosan indicated that it could be applied to dentin, even for direct pulp capping.

Microfluidic experiments could better simulate the dental pulp-dentin complex and take advantage of the dentin barrier [26]. The experiment found that the control group mimicked the attachment relationship between dental pulp cells and dentin. The cells in the phosphoric acid group were more rounded, indicating that the cells might be stimulated by phosphoric acid and had a slight change in shape, but there was no cell apoptosis. EDTA-chitosan and the other materials did not change the cells significantly, proving that EDTA-chitosan was less irritating to the cells than phosphoric acid.

Within the limitations of the present study, it might be concluded that EDTA-chitosan could extrafibrillarly demineralize the collagen fibers, which was more conducive to the preservation of minerals inside the collagen fibers.

Dentin conditioned by EDTA-chitosan could retain part of the smear layer and form a mixed layer and resin protrusions. The bonding strength and durability were better than those of the commonly used phosphoric acid wet bonding and equivalent to SE-Bond in clinical practice. In addition, the direct cytotoxicity of EDTA-chitosan was lower than that of phosphoric acid and SE-Bond, and the indirect cytotoxicity was lower than that of phosphoric acid. EDTA-chitosan had better chelating efficiency, bonding strength, and durability than chitosan. In short, EDTA-chitosan has the potential to be applied to dentin resin for direct bonding restoration and has good clinical application prospects. Future studies should focus on improving the material synthesis method and developing EDTA-chitosan with a stronger chelating ability to shorten the time of the operation and lay the foundation for its clinical application.

Declarations

Ethics approval Approval was obtained from the ethics committee of School and Hospital of Stomatology, Peking University. The procedures used in this study adhere to the tenets of the Declaration of Helsinki.

Conflict of interest The authors declare no competing interests.

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