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Effect of matrix metalloproteinase 8 inhibitor on resin–dentin bonds

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ABSTRACT

Objectives. This study investigated the effect of matrix metalloproteinase 8 (MMP-8) on resin–dentin bonds, assessed the mechanical properties of the interfaces over time, and discussed the potential application of MMP-8 inhibitor I (MMP8-I) as a specific MMP-8 inhibitor to be incorporated into dental adhesives.

Methods. The activation and inhibition of MMP-8 was detected by colorimetric assay. After 1 day, 6 months and 1 year of storage of Control, MMP8-I, and chlorhexidine (CHX) groups, the microtensile bond strengths (μTBS) were used to evaluate the bond strength and failure mode distributions, and nanoleakage analysis was used to evaluate the minor scattered silver particles.

Results. Colorimetric assay showed that the activated MMP-8 was enhanced by adhesive procedures, while it was inhibited by the additional treatment of MMP8-I or CHX. Compared with the Control and CHX groups, the MMP8-I group had significantly higher bond strength and the hybrid layer from the MMP8-I-treated dentin exhibited structural integrity of the collagen network and decreased silver nitrate penetration after 1 year of storage.

Significance. MMP-8 inhibition I protects against the degradation of resin–dentin bonds over time, which is better than broad-scale enzyme inhibitor CHX. It shows that MMP8-I may be used in dentistry for preventing collagen degradation within hybrid layers to extend the longevity of resin–dentin bonds.

1. Introduction

The longevity of resin–dentin bonds is consistently a focus of research in contemporary adhesive dentistry due to the reduction of bond strength over time. The bonded interface is mainly damaged by enzymatic degradation of the collagen matrix [1,2]. Matrix metalloproteinases (MMPs) are present in dentin matrices, which belong to a class of zinc- and calcium-dependent endopeptidases [1,3]. They have been shown to be

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related with the collagen degradation in the hybrid layers, which may be due to the low-pH environment, the activity of tissue inhibitors of MMPs (TIMPs), and the interaction with cysteine cathepsins. Collagenase (MMP-8), gelatinases A and B (MMP-2 and MMP-9 respectively), stromelysin-1 (MMP-3), and enamelysin (MMP-20) have been detected in dentin [4–6]. MMP-8 is the major MMP-collagenase in dentin which is capable of cleaving type I collagen into 3/4- and 1/4-length collagen fragments [7]. Type I collagen is an important component in the hybrid layer, thus its breakdown causes the failure of the resin–dentin bond.

Studies have investigated the preservation of collagen fibrils in the dentin extracellular matrix using different matrix metalloproteinase inhibitors [8,9]. The application of chlorhexidine (CHX), a non-specific protease inhibitor of MMP-2, MMP-9 and cysteine cathepsins, has been found to be beneficial in the preservation of the hybrid layer and prevents the decrease of bond strength in vitro and in vivo [10–13]. However, recent studies have shown that CHX has a toxic effect on odontoblast-like cells [14] and stem cells from human exfoliated deciduous teeth [15]. Therefore, it is relevant to find an alternative MMP inhibitor to improve bonding efficacy. Synthetic MMP-8 Inhibitor I (MMP8-I) selectively inhibits MMP-8, which has a tetrahydroisoquinoline ring situated at the entrance to the active site of MMP-8 and a hydroxamate structure that chelates catalytic zinc ions [16]. The purpose of this study was to examine the endogenous enzymatic bond degradation during etch-and-rinse adhesives and investigated whether MMP8-I can prevent enzymatic bond degradation. The null hypotheses tested were (1) that acid etching adhesive procedures cannot extract and activate MMP-8 in dentin, and (2) that the application of the MMP8-I has no effect on the activity of MMP-8 and bond degradation.

2. Materials & methods

2.1. MMP8 activity assay

Upon signing an informed consent, twenty fresh, non-carious, human third molars from 18 to 25 year-old donors with informed consent were collected and the study was approved by the Sun Yat-sen University Research Ethics Committee. Enamel, roots, and residual pulp tissue were removed and coronal dentin blocks were pulverized using a stainless steel hammer [17]. Unless otherwise stated, all steps were carried out at 4 °C. Dentin powder samples (each 1.2 g) were divided into the following treatment groups: Group 1, dentin powder samples were untreated, rinsed with distilled water, subjected to a brief centrifugation, and stored for 24 h in dark conditions; Group 2, dentin powder samples were demineralized in 1 ml of 35% phosphoric acid (PA) for 15 s, rinsed with distilled water, subjected to a brief centrifugation, and stored for 24 h in dark conditions; Group 3, PA-etched samples were washed, and demineralized dentin powder was mixed with 1 ml of Adper Single Bond 2 (SB2, 3 M ESPE, St. Paul, MN, USA) for 24 h in dark conditions; Group 4, PA-etched samples were rinsed, treated with 1 ml of 8 μM MMP8-I (CAS 236403-25-1, Merck KGaA, Darmstadt, Germany) solution for 30 min, then mixed with 1 ml of SB2 for 24 h in dark conditions; Group 5, PA-etched samples were rinsed, treated with 1 ml of 2% CHX (Sigma, St. Louis, MO, USA) solution for 30 min, and then mixed with 1 ml of SB2 for 24 h in dark conditions.

All dentin powder samples were washed 5 times with distilled water. The total protein of the five groups were extracted with 50 mM of Tris-HCl (pH 6.0), containing 5 mM of $CaCl_2$, 100 mM of NaCl, 0.1% Triton X-100, 0.1% non-ionic detergent P-40, 0.1 mM of $ZnCl_2$, 0.02% NaN_3 and ethylenediaminetetraacetic acid (EDTA)-free protease inhibitor cocktail (Roche Diagnostics GmbH, Germany) [18,19]. Supernatants were collected by centrifugation at 2000 × g for 10 min. The total protein concentration of each extract was determined using the Enhanced BCA Protein Assay Kit (Beyotime, Jiangsu, China), and MMP-8 activity was detected in triplicate using a Colorimetric Assay Kit (GENMED, Shanghai, China). According to the manufacturer's instructions, samples for each of the 5 groups were incubated in the supplier-provided assay buffer for 1 h at 37 °C, and the absorbance was read at 412 nm using a microplate reader (Tecan, Infinite 200 Pro, Grödig, Austria).

2.2. Microtensile bond strength test (μTBS)

Sixty non-carious human third molars were collected. The mid-coronal dentin surfaces were exposed using a slow-speed diamond saw (Isomet, Buehler Ltd., Lake Bluff, IL, USA) and ground with 600-grit silicon carbide paper (SiC paper, Buehler Ltd.) for 1 min under water-cooling conditions to create a smear layer. All teeth were etched with 35% phosphoric acid for 15 s, rinsed for 10 s, and dried appropriately. The specimens were randomly divided into 3 experimental groups according to the application of MMP inhibitors. For Group 1, SB2 was applied according to the manufacturer's instructions and light-cured (Control). For Groups 2 and 3, following the application of 8 μM MMP8-I or 2% CHX on acid-etched dentin for 30 s, SB2 was used and light-cured. A composite build-up was made by resin composite (Z350, 3 M ESPE) in shade A2. Following the adhesive procedure, the whole specimen was stored in distilled water at 37 °C for 24 h to ensure sufficient polymerization [20]. The tooth were longitudinally sectioned in mesio-to-distal and buccal-to-lingual directions across the bonded interface by a diamond saw, then 3 resin-bonded sticks from each tooth were collected [21]. The storage medium of artificial saliva was replaced once a week [22]. After 1 day, 6 months and 1 year of storage, 20 sticks from each group were stressed at a crosshead speed of 1 mm/min until failure in a microtensile tester (Bisco, Inc., Schaumburg, IL, USA). Failure modes were evaluated by stereomicroscopy (M250A, Leica, Wetzlar, Germany), and the interfacial ultrastructure was observed under a scanning electron microscope (SEM, Quanta 200, FEI, Eindhoven, Netherlands).

The obtained data were statistically analyzed using two-way ANOVA to determine the effects of the MMP inhibitor and storage time on microtensile bond strength (μTBS). Post-hoc Tukey's test was used for pairwise multiple comparisons among group means. A multilevel multinomial logit model was used to analyze the distribution of failure modes among groups. Statistical significance was set at $\alpha = 0.05$. All data analysis was performed by SPSS 20.0 software package (SPSS, Chicago, IL, USA).

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