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The inhibitory effect of a polymerisable cationic monomer on functional matrix metalloproteinases

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ABSTRACT

Objectives: This study examined the use of methacryloxylethyl cetyl dimethyl ammonium chloride (DMAE-CB) as a potential matrix metalloproteinases (MMPs) inhibitor on both soluble recombinant and dentine matrix-bound endogenous MMPs, meanwhile attempted to determine the effective anti-MMP group of quaternary ammonium methacrylates (QAMs).

Methods: The possible inhibitory effects of five DMAE-CB mass concentrations (0.1%, 0.5%, 1%, 3%, 5%) on soluble rhMMP-9 were measured using a colorimetic assay kit. Methyl methacrylate (MMA) and [2-(methacryloyloxy)ethyl] trimethylammonium chloride (METMAC) were also screened against rhMMP-9 to compare the inhibitory effect with DMAE-CB. Matrix-bound endogenous MMP-activity was evaluated in completely demineralized dentine beams. Thirty beams were randomly divided into three groups ($N = 10$) and respectively placed into 500 μL of calcium- and zinc-containing media (CM; control), 0.2% chlorhexidine or 3% DMAE-CB in CM aged for 30 days. The changes in modulus of elasticity, loss of dry mass and solubilization of collagen peptides were measured via three-point bending, precision weighing and hydroxyproline assay, respectively.

Results: 0.5–5% mass concentrations of DMAE-CB were highly effective ($P < 0.05$) in inhibiting rhMMP-9 varied between 76.56 ± 6.44% and 97.06 ± 3.24%, the inhibitory effect of MMA was much lower than that of METMAC and DMAE-CB at the same concentration ($P < 0.05$). Dentine beams incubated in 3% DMAE-CB showed only a 26.34% decrease in the modulus of elasticity (75.74% decrease in control), a 1.72 ± 1.56% loss of dry mass (29.70 ± 9.12% loss in control), and significantly less solubilized hydroxyproline when compared with the control ($P < 0.05$).

Conclusions: DMAE-CB is effective in inhibiting both soluble recombinant MMPs and matrix-bound dentine MMPs. Quaternary ammonium group is the effective anti-MMP group of QAMs.

Clinical significance: The incorporation of DMAE-CB into dental adhesives has the potential to enhance the durability of dentine bonding and thus increases the longevity of restorations.

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1. Introduction

Aesthetic tooth-coloured restorations combined with an adhesive system are widely used in the clinical practice of prosthodontics due to the excellent aesthetic properties and acceptable immediate bond strength.[1–3] However, the short longevity of tooth-coloured restorations that is reported to be 5.7 years on average[4] has been attributed to the undesirable durability of dentine bonding. The replacement of restorations leads to further loss of dental tissue and costs about 5 billion dollars per year in the US alone.[5] Therefore, improving the bonding stability of dentine–resin interface has been the hot spot in dental research.

Many factors compromise the bonding durability between the resin material and the demineralized dentine matrix, such as hydrolytic degradation by water sorption, incomplete infiltration of resin monomers and collagenolysis by endogenous hydrolases.[6–8] Among them, the hydrolysis of resin-sparse collagen in hybrid layers is considered to be one of the most important factors leading to bonding failure.[9] In the process of enzymatic hydrolysis, matrix metalloproteinases (MMPs), a group of zinc- and calcium-dependent host-derived proteases, have been confirmed to be deeply involved.[9] MMP-2, -8, -9, and -20 that exist in mineralized dentin[10] can be exposed and activated by the acid-etchants used in adhesive bonding systems[11,12] and lactic acid released by oral pathogenic bacteria.[13] The activated collagen-bound MMPs and/or non-collagen-bound MMPs may progressively degrade the uncovered collagen fibrils in bonded dentine, thus leading to bonding failure.[9,14]

In order to protect the integrity of dentine collagen in hybrid layers and improve the bond durability, chlorhexidine (CHX) has been used as a non-specific MMP inhibitor.[15] When used as a therapeutic primer, CHX can prevent the degradation of hybrid layers.[16,17] However, CHX electrostatically binds to demineralized dentine matrix and may slowly diffuse out of dentine collagen matrix via a competitive desorption mechanism in the presence of other cations.[18] With the loss of CHX, the protective effect on bonding interface could reduce after 9 months and severe degradation could be detected in hybrid layers at 18 months.[19] Ongoing research is currently being conducted on identifying other anti-MMP agents, benzalkonium chloride (BAC) and 12-methacryloyloxydodecylpyridinium bromide (MDPB), antibacterial agents containing quaternary ammonium groups, are effective at inhibiting both soluble recombinant MMPs and matrix-bound dentine MMPs.[20,21]

Methacryloxylethyl cetyl dimethyl ammonium chloride (DMAE-CB) synthesized by our group is a polymerizable cationic quaternary ammonium monomer. Similarly to BAC and MDPB, DMAE-CB contains a positive quaternary ammonium group and has efficient antibacterial effect.[22,23] Moreover, the methacryloyl group of DMAE-CB was supposed to polymerize with resin monomers. Incorporation of DMAE-CB into dental adhesives has proved to be an effective strategy to achieve a reliable antibacterial effect without compromising the bonding efficiency and biocompatibility.[24,25] As DMAE-CB is another potent antimicrobial quaternary ammonium monomer like BAC and MDPB, we speculated that DMAE-CB may also have anti-MMP activity.

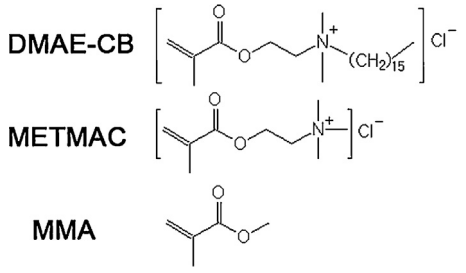

Fig. 1 – The chemical structures of MMA, METMAC and DMAE-CB used in this study.

The mechanism of the anti-MMP action of quaternary ammonium methacrylates (QAMs) is still unsubstantiated up to now. It was hypothesized that the cationic QAMs could electrostatically act on the catalytic site of MMPs, depriving the ability of MMPs to hydrolyze the specific peptide bond of collagen.[21] In this study, we made use of the structure differences between three monomers, methyl methacrylate (MMA), [2-(methacryloyloxy)ethyl] trimethylammonium chloride (METMAC) and DMAE-CB (Fig. 1), to determine whether the positive quaternary ammonium group of QAMs was the effective group for the anti-MMP efficiency.

The objective of this study was to examine the potential of DMAE-CB as an MMP inhibitor and determine the effective anti-MMP group of the monomer. The null hypotheses were that: (1) DMAE-CB does not inhibit soluble MMPs; (2) quaternary ammonium group is not the effective anti-MMP group of quaternary ammonium methacrylates; (3) DMAE-CB has no effect on the endogenous bound MMP activity of demineralized dentine matrices.

2. Materials and methods

2.1. *Inhibition of soluble rhMMP-9*

This assay employed purified recombinant human MMP-9 (ProSpec Inc., Ness Ziona, Israel) and the Sensolyte Generic MMP colorimetric assay kit (AnaSpec Inc., Fremont, CA, USA) for screening anti-MMP activity. The assay kit contains a thiopeptolide that can be cleaved by MMPs to release a sulfhydryl group. The sulfhydryl group reacts with 5,5′-dithiobis (2-nitrobenzoic acid) to produce a coloured reaction product (2-nitro-5-thiobenzoic acid) that can be detected spectrophotometrically at 412 nm.[21]

DMAE-CB was synthesized with further purification and tested at a series of mass concentrations (0.1%, 0.5%, 1%, 3%, 5%). MMA and METMAC were purchased from Sigma–Aldrich (St. Louis, MO, USA) and prepared as 3 wit% solutions for screening. Since the monomer of MMA is difficult to be solubilized in the water and the tested media, MMA and DMSO were first mixed at the proportion of 1:1, and then prepared in distilled water to the final solution. The concentration of DMSO in the tested media was 3%, which was shown to have no effect on the activity of MMP-9 in preliminary study. The chromogenic substrate thiopeptolide solution was diluted to 0.2 mM with the assay buffer in a 1:50 volume ratio. In each