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Orthodontic cement with protein-repellent and antibacterial properties and the release of calcium and phosphate ions

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ABSTRACT

Objectives: White spot lesions often occur in orthodontic treatments. The objective of this study was to develop a novel resin-modified glass ionomer cement (RMGI) as an orthodontic cement with protein-repellent, antibacterial and remineralization capabilities.
Methods: Protein-repellent 2-methacryloyloxyethyl phosphorylcholine (MPC), antibacterial dimethylaminohexadecyl methacrylate (DMAHDM), nanoparticles of silver (NAg), and nanoparticles of amorphous calcium phosphate (NACP) were incorporated into a RMGI. Enamel shear bond strength (SBS) was determined. Calcium (Ca) and phosphate (P) ion releases were measured. Protein adsorption onto specimens was determined by a micro bicinchoninic acid method. A dental plaque microcosm biofilm model was tested.
Results: Increasing the NACP filler level increased the Ca and P ion release. Decreasing the solution pH increased the ion release. Incorporating MPC into RMGI reduced protein adsorption, which was an order of magnitude less than that of commercial controls. Adding DMAHDM and NAg into RMGI yielded a strong antibacterial function, greatly reducing biofilm viability and acid production. Biofilm CFU counts on the multifunctional orthodontic cement were 3 orders of magnitude less than that of commercial control ($p < 0.05$). These benefits were achieved without compromising the enamel shear bond strength ($p > 0.1$).
Conclusions: A novel multifunctional orthodontic cement was developed with strong antibacterial and protein-repellent capabilities for preventing enamel demineralization.
Clinical significance: The new cement is promising to prevent white spot lesions in orthodontic treatments. The method of incorporating four bioactive agents may have wide applicability to the development of other bioactive dental materials to inhibit caries.

1. Introduction

Orthodontic treatments with fixed appliances often cause white spot lesions in enamel, resulting from local biofilm accumulation and acid demineralization [1]. Fluoride (F) therapy *via* fluoridated dentifrices, fluoridated oral rinses and topical F application can be a valuable method for preventing white spot lesions [1,2]. However, these measures depend on patient compliance and therefore are unreliable [1,2]. Hence, preventive measures that do not depend on patient compliance would be more effective. Various resin-based materials have been used in dentistry in composites, cements and adhesives [3–6]. Resin-modified glass ionomer cements (RMGIs) possess desirable F-releasing capability and clinically acceptable enamel bond strengths, therefore have been used as orthodontic cements [5,6]. However, white spot lesions around orthodontic brackets are still common, jeopardizing the health and esthetics of the teeth, and indicating that F alone cannot prevent enamel

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demineralization [1,2]. Hence, it is beneficial to incorporate antimicrobial agents into RMGI to enhance the antibacterial ability to reduce biofilm acids [7].

Quaternary ammonium methacrylates (QAMs) are effective anti-biofilm agents [8,9]. QAMs have been incorporated into dental materials including composites, bonding agents and RMGI, achieving strong antibacterial functions [10–13]. The antibacterial potency of QAMs was shown to increase with increasing the alkyl chain length (CL) of the ammonium groups [14]. Recently, dimethylaminohexadecyl methacrylate (DMAHDM) with a CL of 16 was synthesized and incorporated into composites and bonding agents, producing a strong antibacterial activity [15,16]. However, the antibacterial activity of QAM in an orthodontic cement is limited to the material's surface due to the "contact-inhibition" mechanism [13]. It is beneficial for the orthodontic cement to kill not only the biofilms on its surface, but also the nearby biofilms away from its surface, because plaque buildup in the vicinity of the bracket can still produce acids to cause white spot lesions. Silver (Ag) is antibacterial against a wide range of microorganisms [17,18]. Previous studies developed dental resins containing nanoparticles of silver (NAg) with a potent antibacterial activity [19–21]. NAg resins can inhibit biofilms away from the resin surface due to the release of Ag ions [17,18]. NAg in a RMGI indeed inhibited biofilms away from its surface, without compromising the color of RMGI because of the low NAg concentration [21]. Therefore, the present study combined DMAHDM with NAg in RMGI for the first time as an orthodontic cement to enhance its antibacterial potency and inhibit adherent biofilms as well as biofilms away from its surface.

Another approach is to develop protein-repellent materials. RMGIs accumulated more bacteria than other orthodontic cements, due to the relatively rough surfaces, high surface-free energy and polarity of RMGIs [22,23]. Salivary proteins are known to act as receptors for bacterial adhesion [24,25]. Hence, it would be desirable to develop a RMGI that can repel protein adsorption, thereby reducing bacterial adhesion. 2-methacryloyloxyethyl phosphorylcholine (MPC) is a methacrylate with a phospholipid polar group in the side chain [26]. It was found that MPC could resist protein adsorption and bacterial adhesion [26,27]. Recently, MPC was incorporated into dental composite, dentin bonding agent and RMGI, achieving a strong protein-repellent ability.[15,16,28]

Previous studies showed that RMGI had little demineralization-inhibiting effect, because the low-pH environment due to oral biofilms inhibits the remineralization [5,6]. Therefore, it is beneficial to incorporate remineralization agents into RMGI to enhance the remineralization capability of RMGI. Nanoparticles of amorphous calcium phosphate (NACP) were synthesized and incorporated into composites and adhesives [29–32]. These NACP composites and adhesives showed effective remineralization and caries-inhibition [29–32]. However, there has been no report on the incorporation of NACP into a RMGI. Furthermore, there has been no report on the development of a protein-repellent, antibacterial and remineralizing RMGI as an orthodontic cement for preventing white spot lesions.

The objective of this study was to develop a novel RMGI orthodontic cement with protein-repellent, antibacterial and remineralization capabilities for the first time. The following hypotheses were tested: (1) Incorporation of MPC, DMAHDM, NAg, and NACP into RMGI would not compromise the enamel bond strength; (2) RMGI containing NACP would be "smart" and could increase the calcium and phosphate ion release at cariogenic pH, when these ions would be most needed to combat demineralization; and (3) RMGI containing MPC, DMAHDM, NAg, and NACP would greatly reduce protein adsorption, biofilm growth and lactic acid production, compared to commercial orthodontic cements.

2. Materials and methods

2.1. MPC incorporation into RMGI

A RMGI (Vitremer, 3 M, St. Paul, MN; referred to as VT) consisted of fluoroaluminosilicate glass particles and a light-sensitive, aqueous polyalkenoic acid. Indications include Class III, V and root-caries restoration, Class I and II in primary teeth, and core-buildup. A powder/liquid mass ratio of 2.5/1 was used according to the manufacturer. VT was selected because RMGIs have been used as orthodontic bracket-bonding cements [33,34]. The purpose was to investigate a model system, and then the method of incorporating MPC, DMAHDM, NAg, and NACP can be applied to other orthodontic cements.

MPC was obtained commercially (Sigma-Aldrich, St. Louis, MO) which was synthesized *via* a method reported by Ishihara et al. [26] The MPC powder was mixed with VT at MPC/(VT+MPC) mass fraction of 3%. A previous study showed that the incorporation of 3% MPC yielded a strong protein-repellent capability without compromising the enamel bond strength.[28]

2.2. NAg incorporation into RMGI

Silver 2-ethylhexanoate (Strem, Newburyport, MA) of 0.1 g was dissolved into 0.9 g of 2-(*tert*-butylamino)ethyl meth-acrylate (TBAEMA, Sigma-Aldrich) [19,20]. TBAEMA improved the solubility by forming Ag-N bonds with Ag ions to facilitate the Ag salt to dissolve in the resin solution [19,20]. TBAEMA contains reactive methacrylate groups which can be chemically bonded in the resin upon photo polymerization. The Ag solution was incorporated into VT at a silver 2-ethylhexanoate/(VT + silver 2-ethylhexanoate) mass fraction of 0.1%. This mass fraction was selected based on previous studies showing a strong antibacterial activity without compromising mechanical properties [19,20].

2.3. DMAHDM incorporation into RMGI

DMAHDM was synthesized using a modified Menschutkin reaction where a tertiary amine was reacted with an organo-halide [35]. A benefit of this reaction is that the reaction products are generated at virtually quantitative amounts and require minimal purification [35]. Briefly, 10 mmol of 2-(dimethylamino) ethyl methacrylate (DMAEMA, Sigma-Aldrich, St. Louis MO) and 10 mmol of 1-bromohexadecane (BHD, TCI America, Portland, OR) were combined with 3 g of ethanol in a 20 mL scintillation vial. The vial was stirred at 70 °C for 24 h. The solvent was then removed *via* evaporation to yield DMAHDM [35]. The DMAHDM was mixed with VT at a DMAHDM/(VT + DMAHDM) mass fraction of 1.5%. DMAHDM mass fractions $\geq$ 2% were not used due to a decrease in the enamel bond strength when DMAHDM was combined with 3% MPC and 0.1% NAg in RMGI in preliminary study [21].

2.4. NACP incorporation into RMGI

NACP was synthesized using a spray-drying technique [29]. Briefly, calcium carbonate and dicalcium phosphate were dissolved in acetic acid to produce Ca and P concentrations of 8 mmol/L and 5.333 mmol/L, respectively. The Ca/P molar ratio for the solution was 1.5, the same as that for ACP. The solution was sprayed into a heated chamber of a spray-drying apparatus. The dried particles were collected *via* an electrostatic precipitator (Air Quality, Minneapolis, MN), yielding NACP with a mean particle size of 116 nm.

To mix NACP into RMGI, a resin was added to RMGI so that the mixed paste was cohesive and not dry. The resin consisted of BisGMA (bisphenol A glycidyl dimethacrylate) and TEGDMA