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Effect of charge density of bonding agent containing a new quaternary ammonium methacrylate on antibacterial and bonding properties

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ABSTRACT

Objective. Quaternary amine charge density is important because when the negatively charged bacteria contact the positive quaternary amine charge, the electric balance is disturbed and the bacterium could be disrupted. There has been no report on the effects of charge density on the antibacterial efficacy of dental bonding agents. The objective of this study was to synthesize a new quaternary ammonium methacrylate, and investigate the effects of charge density of bonding agent on bacteria early-attachment, biofilm colony-forming units (CFU) and dentin bond strength.

Methods. Dimethylaminododecyl methacrylate (DMAHDM) with an alkyl chain length of 16 was synthesized and mixed into Scotchbond Multi-Purpose adhesive and primer (SBMP) at mass fractions of 0%, 2.5%, 5%, 7.5%, and 10%. A microtensile dentin bond test was performed. The density of quaternary ammonium groups was measured using a fluorescein dye method. *Streptococcus mutans* (*S. mutans*) early-attachment was examined at 4 h, and biofilm colony-forming units (CFU) were measured at 2 days.

Results. All groups had similar microtensile bonding strengths (mean ± sd; $n = 40$) of about 60 MPa ($p > 0.1$). Quaternary amine charge density of bonding agents monotonically increased with increasing DMAHDM mass fraction. Bacteria early-attachment coverage greatly decreased with increasing DMAHDM content in the resin. Biofilm CFU at 10% DMAHDM was reduced by more than 4 log, compared to SBMP control. Charge density of bonding agent was inversely proportional to bacteria early-attachment coverage and biofilm CFU.

Significance. Increasing the quaternary amine charge density of dentin bonding agent resin was shown to greatly reduce *S. mutans* attachment and decrease biofilm CFU by four orders of magnitude, without compromising the dentin bond strength. The new DMAHDM is promising for use in bonding agents and other antibacterial restorative materials to inhibit caries.

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1. Introduction

Secondary (recurrent) caries at the tooth-restoration margins has been suggested in previous studies as one of the primary reasons for restoration failure [1–4]. The replacement of failed restorations accounts for 50% or more of all the restorations performed [5,6], costing tens of billions of dollars annually. For example, the annual cost for tooth cavity restorations was approximately $46 billion in 2005 in the United States [7]. Furthermore, the need for tooth restorations is increasing rapidly with an aging population, longer life expectancy, and increased tooth retention in seniors [8]. Oral biofilms produce organic acids and enzymes which can lead to caries [1,9]. In addition, while resin composites are the principal material for cavity restorations [4,10–16], resins not only have no antibacterial function, but also may even accumulate more biofilms/plaques in vivo than other restorative materials [17,18]. Therefore, there is a need to develop antibacterial dental resins to inhibit biofilms and caries.

In previous studies, antibacterial resins containing quaternary ammonium methacrylates (QAMs) were synthesized [19–23]. 12-Methacryloyloxydodecyl-pyridinium bromide (MDPB) could be copolymerized and covalently bonded in resins, thus becoming immobilized and exerting a contact-killing capability against oral bacteria and biofilms [24,25]. Several other antibacterial materials were recently reported, including a methacryloxylethyl cetyl dimethyl ammonium chloride (DMAE-CB)-containing adhesive [26], antibacterial glass ionomer cements [27], and antibacterial nanocomposites and bonding agents using a quaternary ammonium dimethacrylate (QADM) [28–30].

Bonding agents are used to adhere the restorations to tooth structures [31,32]. Extensive studies have improved the tooth-restoration bond strength and the understanding of the nature of adhesion [33–38]. Rendering the bonding agent antibacterial is meritorious in order to combat biofilm acids and recurrent caries at the tooth-restoration margins [24–26,39]. Besides residual bacteria in the prepared tooth cavity, marginal leakage would allow new bacteria to invade the tooth-restoration interface. Antibacterial bonding agents could help inhibit the residual as well as the invading bacteria [19,24,25]. Efforts were made to make both the primer and the adhesive resin to be antibacterial [19,26,29,30,40,41]. Regarding the antibacterial mechanism, quaternary ammonium salts (QAS) can cause bacteria lysis by binding to cell membrane to cause cytoplasmic leakage [39,42]. When the negatively charged bacteria contact the positive quaternary amine charge (N^+), the electric balance is disturbed and the bacterium could explode under its own osmotic pressure [39,42]. Hence, the quaternary amine charge density is an important factor in the antibacterial efficacy. In a previous study, the charge density of poly(4-vinyl-N-alkylpyridinium bromide) coated glass slide was calculated as an index of surface properties [43]. Another study showed that the charge density of a dental resin increased with increasing the quaternary ammonium dimethacrylate mass fraction [21]. Although charge density evaluation was included in these reports, their main purpose was not to correlate these findings specifically with the efficiency of the antimicrobial agent. However, to date, the effects of quaternary amine charge density on the antibacterial efficacy and dentin bond strength of dental bonding agents have not been reported.

The objectives of this study were to: (1) synthesize a new QAM for incorporation into a dental bonding agent; and (2) systematically investigate the effects of quaternary amine charge density of bonding agent on bacteria early-attachment, biofilm colony-forming units (CFU) and dentin bond strength. The new QAM was incorporated into a primer and an adhesive at a series of mass fractions, thus allowing the quaternary amine charge density on the cured resin surface to be systematically varied. It was hypothesized that: (1) Increasing the charge density on bonding agent will monotonically decrease bacteria early-attachment; (2) Increasing the charge density of bonding agent resin will reduce the biofilm CFU; (3) Adding the QAM into bonding agent will not compromise the dentin bond strength, compared to the control without QAM.

2. Materials and methods

2.1. *Development of new QAM and antibacterial bonding agent*

Dimethylaminododecyl methacrylate (DMAHDM) with an alkyl chain length of 16 was synthesized using a modified Menschutkin reaction [21,28], where a tertiary amine group was reacted with an organo-halide. A benefit of this reaction is that the reaction products are generated at virtually quantitative amounts and require minimal purification [21]. Briefly, 10 mmol of 1-(dimethylamino)docecane (Sigma, 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, yielding DMAHDM as a clear, colorless, and viscous liquid. Details of this method have been described recently [21,28,44].

Scotchbond Multi-Purpose adhesive and primer (referred as "SBMP") (3M, St. Paul, MN) were used as the parent bonding system to test the effect of incorporation of antibacterial agent. According to the manufacturer, SBMP adhesive contained 60–70% of bisphenol A diglycidyl methacrylate (Bis-GMA) and 30–40% of 2-hydroxyethyl methacrylate (HEMA), tertiary amines and photo-initiator. SBMP primer contained 35–45% of HEMA, 10–20% of a copolymer of acrylic and itaconic acids, and 40–50% water. DMAHDM was mixed with SBMP primer at DMAHDM/(SBMP primer + DMAHDM) mass fraction of 2.5%, 5%, 7.5%, and 10%. Similarly, DMAHDM was mixed with SBMP adhesive at DMAHDM/(SBMP adhesive + DMAHDM) mass fraction of 2.5%, 5%, 7.5%, and 10%. The 10% mass fraction followed previous studies [29,30]. Therefore, five groups were tested:

(1) Unmodified SBMP primer and adhesive (referred to as "SBMP control");
(2) SBMP primer + 2.5% DMAHDM, SBMP adhesive + 2.5% DMAHDM ("SBMP + 2.5% DMAHDM");
(3) SBMP primer + 5% DMAHDM, SBMP adhesive + 5% DMAHDM ("SBMP + 5% DMAHDM");