DENTAL MATERIALS XXX (2017) XXX–XXX

Available online at www.sciencedirect.com

ScienceDirect

journal homepage: www.intl.elsevierhealth.com/journals/dema

Antibiofilm properties of model composites containing quaternary ammonium methacrylates after surface texture modification

Guilherme Ferreira Rego[a], Marina Lermen Vidal[a], Gil Mendes Viana[b], Lucio Mendes Cabral[b], Luis Felipe Jochims Schneider[a,c], Maristela Barbosa Portela[a], Larissa Maria Cavalcante[a,c,d,*]

[a] School of Dentistry, Federal Fluminense University - UFF, Niterói, RJ, Brazil
[b] School of Pharmacy, LabTIF, Federal University of Rio de Janeiro - UFRJ, Ilha do Fundão, Rio de Janeiro, RJ, Brazil
[c] Nucleus for Dental Biomaterials Research, UVA-Veiga de Almeida University, Rio de Janeiro, RJ, Brazil
[d] School of Dentistry, UNIVERSO-Salgado de Oliveira University, Niterói, RJ, Brazil

ARTICLE INFO

Article history:
Received 16 May 2017
Received in revised form
21 June 2017
Accepted 11 July 2017
Available online xxx

Keywords:
Model composites
Surface roughness
Quaternary ammonium methacrylate
Biofilm inhibition
Toothbrush abrasion

ABSTRACT

Objective. Investigate antimicrobial properties and surface texture of model composites with different concentration and alkyl chain length of quaternary ammonium monomers (QAS).
Methods. Monomers derived from QAS salts with alkyl chain lengths of 12 carbons ((dimethylaminododecyl methacrylate) DMADDM) and 16 carbons (dimethylaminohexadecyl methacrylate—DMAHDM) were obtained from the reactions of their respective organo-halides with the tertiary amine 2-(dimethylamino)ethyl methacrylate (DMAEMA). DMADDM and DMAHDM were incorporated into model composite in concentrations of 5 or 10%, resulting the following groups: G12.5 (DMADDM 5%), G12.10 (DMADDM 10%), G16.5 (DMAHDM 5%), G16.10 (DMAHDM 10%) and GC (control). Biofilm viability, lactic acid production and surface roughness were analysed 24 h after samples preparation (initial), repeated after toothbrush abrasion and after polishing simulation. Data were submitted to ANOVA and Tukey's test ($p \leq 0.05$).
Results. The longer the molecular chain size of QAS and the higher its concentration (G16.10), the lower was the viability and the production of lactic acid by the biofilm. No differences were detected in initial roughness' measurements among groups. However, after abrasion, there was an increase of biofilm viability and lactic acid production. Composites containing QAS presented rougher surfaces compared to the CG. After polishing, biofilm viability and surface roughness were statistically similar for all groups. Nevertheless, DMAHDM at 10% showed reduction in lactic acid production.
Significance. Chain length and concentration of QAS influenced biofilm development and production of lactic acid. Longer chains and higher concentrations of QAS promoted better antimicrobial properties. Changes in surface texture caused by abrasion, decreased antibiofilm properties.

* *Corresponding author at*: Universidade Federal Fluminense, School of Dentistry, Rua Mario Santos Braga, 28, Campus do Valonguinho, Centro Niterói, RJ CEP: 24020-140, Brazil.
E-mail address: lara_cavalcante@yahoo.com.br (L.M. Cavalcante).
http://dx.doi.org/10.1016/j.dental.2017.07.010
0109-5641/

1. Introduction

Secondary caries stand out as one of the main reason for failure in the use of dental composite restorations [1–5]. The biofilm formed at restorations can, through the metabolism of carbohydrate and the formation of acids in the biofilm accumulated at the interface of restorations cause secundary caries [6–9]. Furthermore, the unreacted monomers from the resin matrix stimulate bacterial growth [7,9].

The development of recurrent caries is a multifactorial issue. Features related both to the patient and to the restorative procedure will dictate the propensity of secondary caries formation. Opdam et al. observed that the risk of development of secondary caries is higher in patients with medium and high decay rate, and the greater the number of involved faces, the higher is the probability of failure (about 30–40% higher) [10]. The formation of secondary caries is also related to the marginal dental substrate and the cavity's depth, so that margins in dentine and deeper lesions are more prone to recurrent caries [11]. Its histopathology, however, is not different from primary caries, which is a localized process of both demineralization of dentin and enamel caused by biofilm [12].

One alternative to control biofilm growth and consequent development of a new cavity is the incorporation of methacrylic monomers derived of quaternary ammonium salts (QAM) to the organic matrix of resin composites [13–16]. These monomers cause a disturbance in the electrical balance of the bacterial membrane through the contact of their positively charged molecules with the negatively charged cell, causing rupture of its membrane and cell death [13,15–17]. According to some studies, quaternary ammonium methacrylates also exhibit low cytotoxicity to cells of the oral cavity about twenty times lower than the BIS-GMA, a monomer widely used in dental composites available on the market [14,18].

Among the monomers derived from quaternary ammonium salts dimethylaminododecyl methacrylate (DMADDM) and dimethylaminohexadecyl methacrylate (DMAHDM) stand out [13,14,17–19]. They are generally incorporated in concentrations of 5% and 10% by mass in adhesives and resin composites [13,19–21]. The molecules of DMADDM and DMAHDM have an amine core, a methacrylate termination and a long chain of hydrocarbons (12 alkyl chain to DMADDM and 16 to DMAHDM). The amine nucleus is responsible for the positive charge of the molecule and consequent antimicrobial action; the methacrylate termination ensures the incorporation of the monomer to the polymer chain, allowing inhibitory action in the long term [13,18,22]. Regarding the hydrocarbon chain, studies show the relationship between chain length and bactericidal action, so that longer chains promote increased hydrophobicity, leading to greater penetration into the bacterial membrane and consequent inhibition of biofilm [13,16,17].

Besides promising, the studies available in the literature do not simulate clinical conditions, such as finishing and polishing or toothbrush abrasion of composites containing QAM, which may affect the antibiofilm properties of these materials. Therefore, the objective of this study was to determine the effect of concentration and chain length of the quaternary ammonium methacrylates on (a) viability of induced *Streptococcus mutans*, (b) the formation of lactic acid on composites and (c) surface roughness of novel resin composites after toothbrush abrasion and polishing.

The study hypothesis was that the higher the concentration and chain length of quaternary ammonium, the lower the viability of the biofilm induced on the composite; the lower the formation of lactic acid by *S. mutans* on composites; the lower the surface roughness of the resin after toothbrush abrasion.

2. Materials and methods

2.1. Synthesis of antimicrobial monomers

The antimicrobial monomers were synthesized using the Menschutkin reaction, as previously reported [23]. In this addition reaction, a tertiary amine and an organo-halide were added, in equal amounts (60 mmol), into a round bottom flask coupled to a condenser with 20 ml of ethanol and were refluxed for 24 h. After that, the solvent was evaporated to dryness (rotary evaporator) to afford the pure monomer, which did not require any purification. For each monomer, a different organo-halide was used (Scheme 1), since the tertiary amine was always 2-(dimethylamino)ethyl methacrylate (DMAEMA).

To characterize the reaction products, Nuclear Magnetic Resonance (NMR) Spectroscopy and High-Resolution Mass Spectrometry (HRMS) were used. ^{1}H and ^{13}C NMR spectra were recorded on an Avance 200 MHz spectrometer (Bruker, Billerica, MA, USA), using $CDCl_3$ as the solvent. Standard Bruker software was used throughout and chemical shifts were given in ppm (δ scale) and coupling constants (*J*) were given in hertz (Hz). High-resolution mass spectra were obtained on a Bruker microTOF II mass spectrometer using ESI.

2.1.1. Dimethylaminododecyl methacrylate (DMADDM)

Yield 24.07 g (98.8%); ^{1}H NMR ($CDCl_3$) δ 6.11 (s, 1H), 5.64 (s, 1H), 4.64 (br s, 2H), 4.11 (br s, 2H), 3.72–3.50 (m, 2H), 3.47 (s, 6H), 1.92 (s, 3H), 1.81–1.63 (m, 2H), 1.30–1.17 (m, 18H), 0.84 (t, $J = 6.6$ Hz, 3H); ^{13}C NMR ($CDCl_3$) δ 166.4, 135.3, 127.4, 65.6, 62.4, 58.3, 52.0, 32.0, 29.6, 29.5, 29.5, 29.4, 29.3, 26.4, 23.0, 22.7, 18.3, 14.2; HRMS-ESI: *m/z* $[M-Br]^+$ calculated for $C_{20}H_{40}NO_2Br$: 326.3059; found: 326.3062.

2.1.2. Dimethylaminohexadecyl methacrylate (DMAHDM)

Yield 27.52 g (99.3%); ^{1}H NMR ($CDCl_3$) δ 6.12 (s, 1H), 5.65 (s, 1H), 4.64 (br s, 2H), 4.13 (br s, 2H), 3.70–3.55 (m, 2H), 3.49 (s, 6H), 1.93 (s, 3H), 1.90–1.61 (m, 2H), 1.37–1.20 (m, 26H), 0.85 (t, $J = 5.7$ Hz, 3H); ^{13}C NMR ($CDCl_3$) δ 166.4, 135.3, 127.4, 65.6, 62.3, 58.3, 52.0, 32.0, 29.7, 29.6, 29.5, 29.4, 29.3, 26.4, 23.0, 22.7, 18.3, 14.2; HRMS-ESI: *m/z* $[M-Br]^+$ calculated for $C_{24}H_{48}NO_2Br$: 382.3685; found: 382.3691.

2.2. Formulation of composites with antimicrobial action

For each material, Bis-GMA and TEGDMA (Esstech Inc., USA, batch TSMPOO4397) were used in a 1:1 ratio by weight, 1% camphorquinone (Esstech Inc., USA; batch TSNP004397) and 1% of amine EDMAB (Sigma–Aldrich, USA, batch MKBB3614). Furthermore, different concentrations (5% or 10%) of the

Please cite this article in press as: Rego GF, et al. Antibiofilm properties of model composites containing quaternary ammonium methacrylates after surface texture modification. Dent Mater (2017), http://dx.doi.org/10.1016/j.dental.2017.07.010