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.

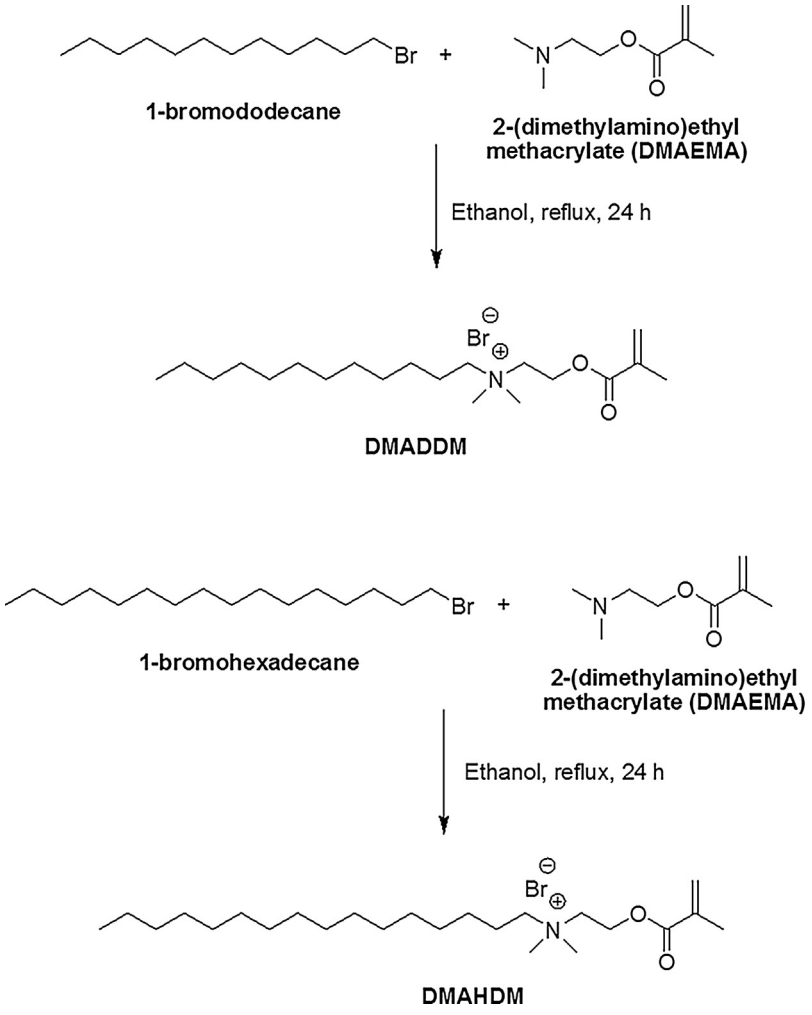
2.1.2. Dimethylaminohexadecyl methacrylate (DMAHDM)

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



Scheme 1 – Synthesis of antimicrobial monomers DMADDM and DMAHDM.

Table 1 – Division of groups according to the monomer employed and its concentration.

Groups	Antibiofilm monomer
Group 12.5	DMADDM 5%
Group 12.10	DMADDM 10%
Group 16.5	DMAHDM 5%
Group 16.10	DMAHDM 10%
Group C	Control

antibacterial monomers listed above were also incorporated into the resin matrix. Inorganic filler particles were added in order to obtain a composite containing 50% mass load. Regarding the inorganic content, 15% of 40 nm pre-silanized spherical silica particles (Aerosil R 812, batch 3523102) and 85% of 0.7 μm pre-silanized borosilicate barium glass particles (V-258-4107, batch 699-17, Esstech Inc.) were incorporated into the composite. Thus, four resin composites were formulated, containing different antimicrobial agents in different concentrations, and one control group without antibacterial action (Table 1).

2.3. *Specimens preparation*

The experimental composites were bulk-filled in a metal mold with dimensions of 8 mm diameter and 2 mm thickness. A metal weight of 65 g was placed over a mylar strip and a glass slide for 30 s to plan the sample and remove the excess of resin. Subsequently, the samples were photoactivated with a LED light source (LED Demi, Kerr, Wisconsin, USA) for 40 s on each side, at an irradiance of 800 mW/cm^2 checked with a radiometer (LED Radiometer, Demetron SDS Kerr, Wisconsin, EUA) at the end of preparation of each specimen. Ten specimens were prepared (n = 10) for each experimental group to assess surface roughness, gloss retention, and of biofilm growth inhibition. At the end of this step, one side of the specimen was standardized so the readings and aging procedures could be performed.

2.4. *Induction of biofilm*

The mature biofilm used was composed exclusively of *S. mutans*. This microorganism was selected because it is one of the main microorganisms involved in caries initiation and progression.

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

Specimens were exposed to ultraviolet light in a laminar flow cabinet for 30 min to be sterilized. After this step, put in wells of sterile culture cells plates.

Isolates of "American Type Culture Collection" (ATCC 25175, Fundação Oswaldo Cruz, Rio de Janeiro, Brazil) were used for the induction of *S. mutans* biofilm on the specimens. *S. mutans* aliquots are stored in liquid nitrogen (approximately −180 °C). For each experiment, a single aliquot was thawed.

Aliquots of cell suspension with 2×10^5 cells/ml in a culture medium supplemented with 2% sucrose were inoculated onto the specimens and kept for 48 h at 37 °C in microaerophilic conditions.

2.5. Analysis of metabolic activity (lactate concentration)

After a 48 h incubation period, the specimens were rinsed with cysteine peptone water (CPW) to remove loose cells and transferred to new sterile plates containing 1.5 ml of buffered peptone water solution (BPW) supplemented with 0.2% sucrose (v/v). To allow the acid formation, the plates were incubated anaerobically for 3 h at 37 °C. After this period, the lactate concentration in the BPW solution was determined using standard enzymatic method of lactate dehydrogenase [24].

2.6. Analysis of bacterial viability

A colorimetric analysis of [3-(4,5-dimethylthiazol-2-yl)-2,5-diphenyltetrazolium bromide] (MTT) was used, which measures the reduction of MTT to form crystals by metabolically active and viable biofilm cells.

The specimens were inserted in Falcon tubes containing 1 ml of sterile phosphate buffered saline (PBS) and vortexed for 1 min for 3 times to promote detachment of the biofilm from the surface of the specimens. The PBS containing the biofilm was aspirated and transferred to microtubes and then taken to the centrifuge. After centrifugation, the isolated biofilm on the bottom of the microtube was retained and the remaining PBS was aspirated and discarted. 100 μl of MTT was added in each microtube and incubated in microaerophilic conditions at 37 °C for 1 h. After this period, 100 μl of dimethylsulfoxide (DMSO) were added in each microtube and kept for 20 min under stirring and sheltered from the light. Finally, the content of each microtube was transferred to a 96 well plate to be read in a microplate reader at absorbance of 540 nm.

2.7. Surface roughness

The surface roughness was measured using a contact profilometer (SJ-201—Mitutoyo, Japan) using a constant speed of 1.0 mm/s at the applied force of 6 mN. The tip covered a distance of 2 mm and 6 readings were done by specimen, calculating an average to obtain a value for each specimen, given in μm. The parameter used was the Ra (arithmetic mean of peaks and valleys of a surface).

2.8. Toothbrush abrasion

A toothbrush simulator (MEV2, Odeme, São Paulo, Brazil) was used for abrasion on the surface of the specimens. Toothbrushes (Oral-B Indicator® Plus, soft bristles) were coupled to the machine in order to present intimate contact with the surface of the resins. Brushing simulation was done by applying a vertical load of 2.5 N, with horizontal movements. The ratio of distilled water/dentifrice (Colgate Total 12 Clean Mint) used was 1:1. For each specimen, 3 ml of the mixture water/dentifrice ("slurry") were initially added and 1 ml of the mixture was refilled every 1000 cycles. Subsequently, the samples were removed from the machine, washed with tap water and taken to ultrasonic bath for 10 min to remove any remainder of the slurry.

2.9. Polishing of specimens

The specimens were polished (Aropol-e, AROTEC, Brazil) with 600 and 4000 grid sandpaper at 150 rpm for 60 s under water irrigation.

2.10. Statistical analysis

The results will be submitted to two-way analysis of variance (ANOVA) with repeated measures and Tukey's test (95%).

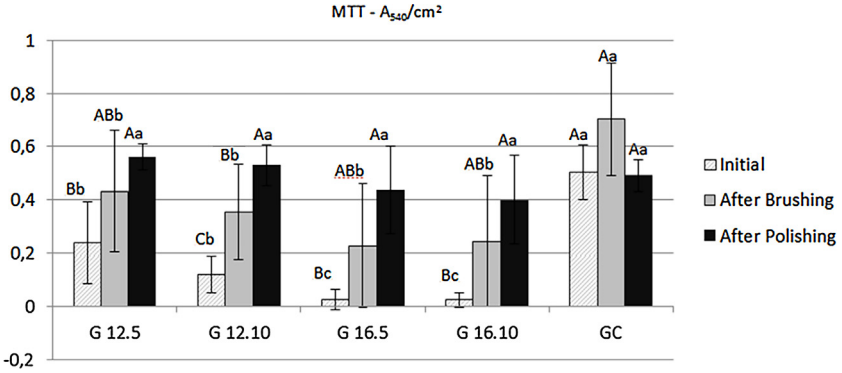


Fig. 1 – Analysis of bacterial viability of composites containing quaternary ammonium and control group. Material p = 0.000; Time p = 0.000; Material × Time p = 0.000; uppercase letters determine analysis by conditions and lowercase letters among materials. Different letters indicate significant statistical difference.

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

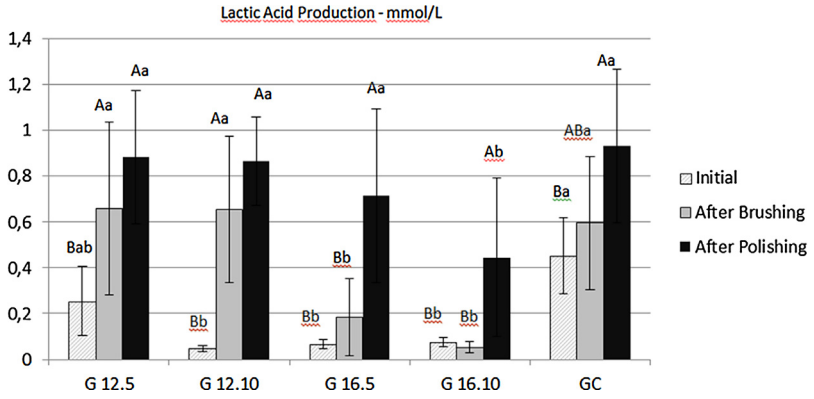


Fig. 2 – Analysis of the metabolic activity of *S. mutans* biofilm induced on composites containing quaternary ammonium and control group. Material p = 0.000; Time p = 0.000; Material × Time p = 0.003; uppercase letters determine analysis by conditions and lowercase letters among materials. Different letters indicate significant statistical difference.

3. Results

The results of bacterial viability are shown in Fig. 1. The groups that had lower initial biofilm viability were G16.5 and G16.10, followed by G12.10 and G12.5. The control group (GC) showed the highest biofilm viability. This initial pattern changed after brushing simulation where all experimental composites containing QAS presented statistically similar losses in inhibitory capacity and the control group maintained the initial default. After polishing, all groups had similar viability values, with no statistical difference.

The results of latic production by biofilm are shown in Fig. 2. Composites containing quaternary ammonium showed lower initial values compared to the control group (GC). Among the groups containing QAS, G12.5 showed the highest initial value. After brushing simulation, G12.5 and G12.10 had an increase in metabolic activity, statistically similar to GC. After polishing, all groups showed high activity of lactate dehydrogenase. Only G16.10 had a statistically different result, compared to the other groups.

Surface roughness measurements in three different stages: initial, after brushing and after polishing were exposed in Fig. 3. No statistical differences were observed among groups for the initial condition. After toothbrush abrasion, G12.5 and G16.10 presented rougher surfaces compared to the control group (GC).

After the polishing procedures, no statistical differences were detected among the groups, and numerical roughness values were similar to the initial condition.

4. Discussion

Dental restorative composites nowadays have high versatility in their clinical indications, being used at anterior and posterior teeth to restore form, function and aesthetics of elements affected by carious lesions, wear and fracture. However, they still have a relatively high failure rate, especially due to secondary caries [1–5]. In this study, methacrylate monomers derived from quaternary ammonium salts (QAS) with antimicrobial properties, were synthesized as dimethylaminododecyl methacrylate (DMADDM) and dimethylaminohexadecyl methacrylate (DMAHDM) and incorporated into experimental composites in concentrations of 5% and 10%.

The initial results of biofilm viability showed that composites containing DMAHDM presented greater inhibition of *S. mutans* than the other evaluated groups. This can be explained

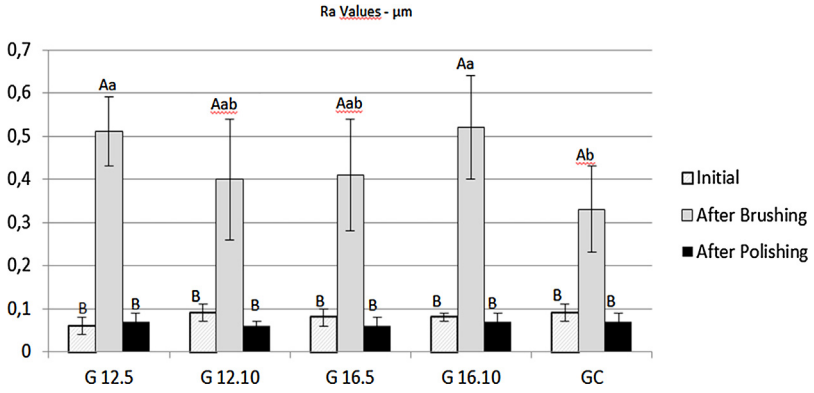


Fig. 3 – Surface roughness of the groups containing QAS and control group. Material p = 0.011; Time p = 0.000; Material × Time p = 0.000; uppercase letters determine analysis by condition and lowercase letters among materials. Different letters indicate significant statistical difference.

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

by the longer alkyl chain present in this monomer compared to DMADDM. Several studies indicate that larger chains increase the hydrophobicity of the molecule and the tendency of penetration into the bacterial membrane, causing its rupture [13,16,17,19]. A higher inhibition was also observed by increasing the concentration of antimicrobial agents. By increasing the concentration from 5% to 10%, the charge density in the resin matrix is also increased, resulting in higher biofilm inhibition capacity [15,17–19]. These charges present in the amine portion of the molecule of QAS promote attraction and interaction with the bacterial membrane and its rupture. In addition, charges could damage bacterial DNA, disturbing the flow of essential ions and disrupting protein activity [17].

Due to the antimicrobial action mechanism of the ammonium quaternary requires an intimate contact with the bacterial cell, in addition to the fact that these monomers do not have antimicrobial action at a distance, the experimental model of formation a mature biofilm was not used. Probably, the use of a mature biofilm model would not have the antibacterial action on the cellular layers farthest from the surface of the resin. Thus, a biofilm of 2 days (48 h) was chosen, which corresponds to the initial stage of biofilm formation and development, in order to evaluate the microorganisms that remained viable even after the initial bacterial adhesion phase. In this experimental model the viability of the cells, that are able to develop a mature biofilm, was measured. This model has widely been reported in studies that evaluated the antimicrobial capacity of restorative materials [25–27].

After brushing and polishing simulations, there was an increase in viable biofilm in contact with samples containing quaternary ammonium monomers. This is due to the positioning of a polyester strip and the weight placed above during specimens' preparation, creating a "matrix-rich" layer in the surface [28–31]. Thus, after abraded by brushing, part of this region has been lost, exposing filler particles (which have no antibiofilm capacity) and reducing the amount of antimicrobial monomers in the composite surface. This fact was even worse after polishing of the specimens, promoting further reduction of the antimicrobial capacity of the composites. For the control group, the initial values were high due to the absence of antimicrobial monomers in its composition. After toothbrush simulation, we observed an increase in viable biofilm, since the composite surface became rougher and uneven, increasing the area available for biofilm adhesion and development [32,33]. After polishing the resin surface, it became smoother and more uniform, and the pattern of viable biofilm was similar to the initial value. Thus, the first hypothesis was accepted, since composites with higher concentrations and longer chains of antimicrobial monomers reduced biofilm formation. However, this property was attenuated after toothbrush abrasion and polishing.

Despite the fact that in vitro experimental models cannot reproduce the clinical situation, they are essencial for evaluating the variables involved with the biofilm formation in association with dental materials. In vitro models of development carious challenge involved single or multispecies biofilms grown on substrates that were incubated in simulated oral conditions. Besides, *S. mutans* are used in the majority of studies [34–36] others microorganism have also been used as *Streptococcus gordonii*, *Streptococcus sobrinus* and *Lactobacillus* [37]. In the present study, *S. mutans* was used because its the most important etiologic agent of caries disease and in order to control in terms of knowing specifically how this specie was sensitive of ammonium quaternary methacrylates. Addionally, the *S. mutans* ATCC 25175 was already used in previous studies that assessed the antimicrobial activity of others incorporated substances in dental materials [34–36]. The use of a common strain among the researches is also important for comparison the results of the studies in the literature.

The metabolic activity of *S. mutans* biofilms on the composite specimens was analysed by methylbromide thiazolyl blue tetrazolium bromide (MTT) reduction assay. MTT is a colorimetric assay that measures the enzymatic reduction of MTT, a yellow tetrazole, to purple formazan. In the present study, this method has been used to quantify *Streptococcus* biofilm metabolically viable cells under different concentrations and types of quaternary ammonium methacrylates. The counting of colony forming units, dry-weight assessments and spectrophotometric assays are techniques used in this type of analysis despite their limitations. The analysis of features such as density, compaction, multilayer structures and growth aspects can provide relevant information concerning biofilms [38]. Also, the extracellular matrix composition influences the biofilm formation. Studies considering the association of different methods must be developed in order to improve the knowledge about *Streptococcus* biofilm other than that from quantitative methods such as the MTT assay. Better understanding of the biofilm features would promote better understanding about the quaternary ammonium methacrylates antibacterial properties. Although, the present study aimed to measure the bacterial viability on the experimental composites and for this purpose, the MTT method has been proved very effective [24,39].

The results of bacterial metabolism by lactic acid production of *S. mutans* biofilm are related to the viability results, since the specimens containing quaternary ammonium promoted the bacteria death and interruption of its metabolism. However, after toothbrush abrasion, there was a reduction of antimicrobial monomers, increasing the viability of the biofilm and the formation of lactic acid. After polishing, the highest results of bacterial metabolism were observed. For the control group, the lactic acid production followed the same pattern of MTT, with similar values found in each of the sampled specimens, increasing after toothbrushing, due to increased surface roughness and consequent bacterial adhesion. Thus, the second hypothesis was partially accepted, whereas composites containing QAS at higher concentration and with longer alkyl chains in fact promoted higher inhibition of bacterial metabolism. This ability was reduced, however, after simulated tooth brushing and polishing.

Regarding roughness measures, all groups presented similar baseline and final values. However, the results were different after tooth brushing, where the control group presented smoother surfaces. The groups containing quaternary ammonium methacrylates showed more susceptibility to surface degradation because of the presence of monomethacrylate monomers in these groups which may weaken the resin matrix [24,40,41], since its long alkyl chain forms weak bonds with other monomers and result in a less complex and dense polymer chain in the final composite [42].

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

Furthermore, the filler particles could easily come off the matrix, increasing the roughness and porosity of the material [31,43,44]. Consequently, the third hypothesis was rejected, since all groups containing QAS showed initial and after polishing results similar to the control group. After brushing, the measures were higher than the control composite's.

As noted above, inhibition of *S. mutans* biofilm was reduced as the surface of composites containing the QAS was abraded and polished. However, it is possible that this property is maintained at the adhesive interface of a restored cavity, since this region is not abraded or polished. This is a crucial area for secondary caries development. Thus, further studies are needed to assess the behavior of these materials in a simulated cavity, and the integrity of its interface with the tooth structure after the abraded surface of the resin.

5. Conclusions

The addition of quaternary ammonium antibacterial monomers with 10% concentration in experimental composites effectively inhibits *S. mutans* compared to the other groups. In general, composites containing QAS with longer alkyl chain length showed greater antimicrobial action. Changes at surface topography through toothbrush abrasion reduced the antibiobilm capacity of the composites containing quaternary ammonium monomers. The groups containing quaternary ammonium methacrylates showed more susceptibility to surface degradation and roughness increase after toothbrush abrasion.

Acknowledgements

LM Cavalcante and LF Schneider would like to acknowledge FAPERJ for Grants E-26/102.215/2013 and E-26/102.225/2013.

REFERENCES

[1] Bourbia M, Ma D, Cvitkovitch DG, Santerre JP, Finer Y. Cariogenic bacteria degrade dental resin composites and adhesives. J Dent Res 2013;92:989–94.
[2] Deligeorgi V, Mjor IA, Wilson NH. An overview of reasons for the placement and replacement of restorations. Prim Dent Care 2001;8:5–11.
[3] Silvani S, Trivelato RF, Nogueira RD, Goncalves Lde S, Geraldo-Martins VR. Factors affecting the placement or replacement of direct restorations in a dental school. Contemp Clin Dent 2014;5:54–8.
[4] Oginni AO, Adeleke AA. Comparison of pattern of failure of resin composite restorations in non-carious cervical lesions with and without occlusal wear facets. J Dent 2014;42:824–30.
[5] Opdam NJ, Bronkhorst EM, Roeters JM, Loomans BA. A retrospective clinical study on longevity of posterior composite and amalgam restorations. Dent Mater 2007;23:2–8.
[6] Fucio SB, Carvalho FG, Sobrinho LC, Sinhoreti MA, Puppin-Rontani RM. The influence of 30-day-old *Streptococcus mutans* biofilm on the surface of esthetic restorative materials—an in vitro study. J Dent 2008;36:833–9.
[7] Busscher HJ, Rinastiti M, Siswomihardjo W, van der Mei HC. Biofilm formation on dental restorative and implant materials. J Dent Res 2010;89:657–65.
[8] Mutluay MM, Zhang K, Ryou H, Yahyazadehfar M, Majd H, Xu HH, et al. On the fatigue behavior of resin–dentin bonds after degradation by biofilm. J Mech Behav Biomed Mater 2013;18:219–31.
[9] Beyth N, Bahir R, Matalon S, Domb AJ, Weiss EI. *Streptococcus mutans* biofilm changes surface-topography of resin composites. Dent Mater 2008;24:732–6.
[10] Opdam NJ, van de Sande FH, Bronkhorst E, Cenci MS, Bottenberg P, Pallesen U, et al. Longevity of posterior composite restorations: a systematic review and meta-analysis. J Dent Res 2014;93:943–9.
[11] Schwendicke F, Kern M, Blunck U, Dorfer C, Drenck J, Paris S. Marginal integrity and secondary caries of selectively excavated teeth in vitro. J Dent 2014;42:1261–8.
[12] Nedeljkovic I, Teughels W, De Munck J, Van Meerbeek B, Van Landuyt KL. Is secondary caries with composites a material-based problem? Dent Mater 2015;31:e247–77.
[13] Zhou H, Weir MD, Antonucci JM, Schumacher GE, Zhou XD, Xu HH. Evaluation of three-dimensional biofilms on antibacterial bonding agents containing novel quaternary ammonium methacrylates. Int J Oral Sci 2014;6:77–86.
[14] Li F, Wang P, Weir MD, Fouad AF, Xu HH. Evaluation of antibacterial and remineralizing nanocomposite and adhesive in rat tooth cavity model. Acta Biomater 2014;10:2804–13.
[15] Li F, Weir MD, Chen J, Xu HH. Effect of charge density of bonding agent containing a new quaternary ammonium methacrylate on antibacterial and bonding properties. Dent Mater 2014;30:433–41.
[16] Ge Y, Wang S, Zhou X, Wang H, Xu HH, Cheng L. The use of quaternary ammonium to combat dental caries. Materials (Basel) 2015;8:3532–49.
[17] Wu J, Zhou H, Weir MD, Melo MA, Levine ED, Xu HH. Effect of dimethylaminohexadecyl methacrylate mass fraction on fracture toughness and antibacterial properties of CaP nanocomposite. J Dent 2015;43:1539–46.
[18] Li F, Weir MD, Fouad AF, Xu HH. Time kill behavior against eight bacterial species and cytotoxicity of antibacterial monomers. J Dent 2013;41:881–91.
[19] Zhang N, Melo MA, Chen C, Liu J, Weir MD, Bai Y, et al. Development of a multifunctional adhesive system for prevention of root caries and secondary caries. Dent Mater 2015;31:1119–31.
[20] Zhang K, Cheng L, Wu EJ, Weir MD, Bai Y, Xu HH. Effect of water-ageing on dentine bond strength and anti-biofilm activity of bonding agent containing new monomer dimethylaminododecyl methacrylate. J Dent 2013;41:504–13.
[21] Wu J, Weir MD, Melo MA, Xu HH. Development of novel self-healing and antibacterial dental composite containing calcium phosphate nanoparticles. J Dent 2015;43:317–26.
[22] Li F, Weir M, Fouad AF, Xu HHK. Effect of salivary pellicle on antibacterial activity of novel antibacterial dental adhesives using a dental plaque microcosm biofilm model. Dent Mater 2014;30:182–91.
[23] Antonucci JM, Zeiger DN, Tang K, Lin-Gibson S, Fowler BO, Lin NJ. Synthesis and characterization of dimethacrylates containing quaternary ammonium functionalities for dental applications. Dent Mater 2012;28:219–28.
[24] Cheng L, Weir MD, Xu HH, Antonucci JM, Kraigsley AM, Lin NJ, et al. Antibacterial amorphous calcium phosphate nanocomposites with a quaternary ammonium dimethacrylate and silver nanoparticles. Dent Mater 2012;28:561–72.
[25] Zhou C, Weir MD, Zhang K, Deng D, Cheng L, Xu HH. Synthesis of new antibacterial quaternary ammonium monomer for incorporation into CaP nanocomposite. Dent Mater 2013;29:859–70.

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

[26] Cheng L, Weir MD, Zhang K, Wu EJ, Xu SM, Zhou X, et al. Dental plaque microcosm biofilm behavior on calcium phosphate nanocomposite with quaternary ammonium. Dent Mater 2012;28:853–62.
[27] Li F, Weir MD, Xu HH. Effects of quaternary ammonium chain length on antibacterial bonding agents. J Dent Res 2013;92:932–8.
[28] Bashetty K, Joshi S. The effect of one-step and multi-step polishing systems on surface texture of two different resin composites. J Conserv Dent 2010;13:34–8.
[29] Nair VS, Sainudeen S, Padmanabhan P, Vijayashankar LV, Sujathan U, Pillai R. Three-dimensional evaluation of surface roughness of resin composites after finishing and polishing. J Conserv Dent 2016;19:91–5.
[30] Antonson SA, Yazici AR, Kilinc E, Antonson DE, Hardigan PC. Comparison of different finishing/polishing systems on surface roughness and gloss of resin composites. J Dent 2011;39(Suppl. 1):e9–17.
[31] Roselino Lde M, Chinelatti MA, Alandia-Roman CC, Pires-de-Souza Fde C. Effect of brushing time and dentifrice abrasiveness on color change and surface roughness of resin composites. Braz Dent J 2015;26:507–13.
[32] Park JW, Song CW, Jung JH, Ahn SJ, Ferracane JL. The effects of surface roughness of composite resin on biofilm formation of *Streptococcus mutans* in the presence of saliva. Oper Dent 2012;37:532–9.
[33] Ionescu A, Wutscher E, Brambilla E, Schneider-Feyrer S, Giessibl FJ, Hahnel S. Influence of surface properties of resin-based composites on in vitro *Streptococcus mutans* biofilm development. Eur J Oral Sci 2012;120:458–65.
[34] Castro DT, Holtz RD, Alves OL, Watanabe E, Valente ML, Silva CH, et al. Development of a novel resin with antimicrobial properties for dental application. J Appl Oral Sci 2014;22:442–9.
[35] de Alencar NA, Fidalgo TK, Cajazeira MR, Maia LC. Influence of the number of adhesive layers on adhesive interface properties under cariogenic challenge using *Streptococcus mutans*. J Adhes Dent 2014;16:339–46.
[36] Hahnel S, Muhlbauer G, Hoffmann J, Ionescu A, Burgers R, Rosentritt M, et al. *Streptococcus mutans* and *Streptococcus sobrinus* biofilm formation and metabolic activity on dental materials. Acta Odontol Scand 2012;70:114–21.
[37] Ferracane JL. Models of caries formation around dental composite restorations. J Dent Res 2017;96:364–71.
[38] Thein ZM, Samaranayake YH, Samaranayake LP. In vitro biofilm formation of *Candida albicans* and non-albicans *Candida* species under dynamic and anaerobic conditions. Arch Oral Biol 2007;52:761–7.
[39] Li F, Weir MD, Chen J, Xu HH. Comparison of quaternary ammonium-containing with nano-silver-containing adhesive in antibacterial properties and cytotoxicity. Dent Mater 2013;29:450–61.
[40] Cheng L, Zhang K, Melo MA, Weir MD, Zhou X, Xu HH. Anti-biofilm dentin primer with quaternary ammonium and silver nanoparticles. J Dent Res 2012;91:598–604.
[41] Zhang K, Melo MA, Cheng L, Weir MD, Bai Y, Xu HH. Effect of quaternary ammonium and silver nanoparticle-containing adhesives on dentin bond strength and dental plaque microcosm biofilms. Dent Mater 2012;28:842–52.
[42] Ferracane JL. Hygroscopic and hydrolytic effects in dental polymer networks. Dent Mater 2006;22:211–22.
[43] Jasse FF, de Campos EA, Lefever D, Di Bella E, Salomon JP, Krejci I, et al. Influence of filler charge on gloss of composite materials before and after in vitro toothbrushing. J Dent 2013;41(Suppl. 5):e41–4.
[44] Roselino Lde M, Cruvinel DR, Chinelatti MA, Pires-de-Souza Fde C. Effect of brushing and accelerated ageing on color stability and surface roughness of composites. J Dent 2013;41(Suppl. 5):e54–61.

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