

Cytotoxicity evaluation, antibacterial effect, and degree of conversion of QAM-containing adhesives

Isadora Martini GARCIA^(a) 
Tatiana Féres ASSAD-LOSS^(b) 
Luis Felipe Jochims SCHNEIDER^(b) 
Fabrício Mezzomo COLLARES^(c) 
Larissa Maria Assad CAVALCANTE^(b) 
Mônica Almeida TOSTES^(b) 

^(a)University of Maryland School of Dentistry,
Department of General Dentistry, Baltimore,
MD, USA.

^(b)Universidade Federal Fluminense – UFF,
School of Dentistry, Graduate Program in
Dentistry, Federal Fluminense University,
Niterói, RJ, Brazil.

^(c)Universidade Federal do Rio Grande do Sul
- UFRGS, School of Dentistry, Laboratory of
Dental Materials, Federal University of Rio
Grande do Sul, Porto Alegre, RS, Brazil.

Declaration of Interests: The authors
certify that they have no commercial or
associative interest that represents a conflict
of interest in connection with the manuscript.

Corresponding Author:

Tatiana Féres Assad-Loss
E-mail: tatianaassad@yahoo.com.br

Abstract: The aim of this study was to evaluate the influence of adding quaternary ammonium methacrylates (QAMs) to experimental adhesives by assessing the degree of conversion (DC), cytotoxicity against keratinocytes and fibroblasts, and antibacterial activity against biofilm formation. Two QAMs were added to an experimental adhesive: dimethylaminododecyl methacrylate bromododecane (DMADDM) or dimethylaminododecyl methacrylate bromohexadecane (DMAHDM) at three concentrations each: 1, 2.5, and 5 wt.%. Experimental adhesive without QAMs (control group) and commercially available Transbond XT Primer (3M Unitek, Monrovia, California, USA) were used for comparisons. The adhesives were tested for DC, cytotoxicity against keratinocytes and fibroblasts, and antibacterial activity against biofilm formation. DC, cytotoxicity against fibroblasts, and antibacterial activity were analyzed using one-way ANOVA and Tukey's multiple comparisons. Cytotoxicity against keratinocytes was evaluated using the Kruskal Wallis and Dunn's post-hoc ($\alpha = 5\%$) tests. Transbond showed lower DC as compared to 5% DMAHDM, 1% DMADDM, and 5% DMADDM ($p < 0.05$). However, all groups presented proper DC when compared to commercial adhesives in the literature. In the evaluation of cytotoxicity against keratinocytes, Transbond induced higher viability than 2.5 wt.% groups ($p < 0.05$). Against fibroblasts, Transbond induced higher viability as compared to 5 wt.% groups ($p < 0.05$). DMAHDM at 5 wt.% reduced biofilm formation when compared to all the other groups ($p < 0.05$). Despite their cytotoxic effect against keratinocytes, gingival fibroblasts showed higher viability. DMAHDM at 5 wt.% decreased *Streptococcus mutans* viability. The incorporation of DMAHDM at 5 wt.% may be a strategy for reducing the development of white spot lesions.

Keywords: Dental Bonding; Dental Caries; Dental Materials;
Quaternary Ammonium Compounds; Anti-Bacterial Agents.

<https://doi.org/10.1590/1807-3107bor-2024.vol38.0001>

Introduction

Restorative dental materials with antimicrobial properties have been developed to prevent recurrent caries.¹ More recently, resins with antimicrobial activity have also been proposed to avoid white spot lesions (WSLs) in patients wearing orthodontic appliances.²⁻⁴ Patients

Submitted: May 5, 2021
Accepted for publication: December 1, 2021
Last revision: January 30, 2023



treated with orthodontic aligners are at a lower risk for developing WSLs thanks to shorter treatment length and easier oral hygiene.⁵ However, excess bonding material for attachments left on the tooth surface may represent a risk for the development of WSLs.⁶ Even though the prior application of adhesive resins (also called “primer” in orthodontics) has been reported as a step that could be set aside during bracket bonding,⁷ a particular indication for these materials could be the promotion of antimicrobial activity to protect enamel against demineralization.² In this context, the incorporation of antimicrobial agents into adhesive resins could be considered a suitable approach because they come into direct contact with enamel surfaces.⁸ Furthermore, the use of restorative materials or orthodontic bonding of accessories containing therapeutic agents may improve resistance to mechanical and acidic challenges in the oral environment.⁹

Among antimicrobial agents, quaternary ammonium methacrylates (QAMs) alone,^{3,10-14} associated with silver nanoparticles,^{1,9,15,16} or with nanoparticles of amorphous calcium phosphate^{4,17,18} have commanded attention because they promote bacterial cell lysis via direct contact¹⁴ and can be copolymerized within the resin matrix.¹¹ Two QAMs – DMADDM^{1,3,12,15,19-21} and DMAHDM^{4,11,13,19,20,22} – have been investigated at different concentrations. Although many studies have chosen to test such monomers at concentrations from 3 to 10 wt.% with adequate results in mechanical tests and antibacterial activity,^{19,20,23} cytotoxicity may be a limiting factor¹⁰. The release of antibacterial agents into the surrounding site has several disadvantages: a decrease in the mechanical properties of the parental resin-based material over time, short-term effectiveness, and possible cytotoxicity.²⁴ QAMs with different aliphatic chains have been previously tested against human fibroblasts and odontoblasts.²⁵ The materials were tested directly in contact with the cells and eluates from polymerized samples. The tests were performed in an MTT assay with serial dilution. Currently, the use of a test with better predictive power than the MTT, such as the sulforhodamine B (SRB) assay, has been advocated.²⁶ Furthermore, other cells such

as keratinocytes could be evaluated, given their possible contact with resins in the region close to the gingiva.

DMADDM at 1.5 wt.%, 3 wt.%, and 5 wt.% had already been added to a commercial adhesive, and a remarkable reduction in the metabolic activity of microorganisms was observed at 3 wt.%.³ In another study, when DMADDM was tested at 2.5 wt.% and 5 wt.%, incorporation of 5 wt.% showed higher antibacterial activity against mature biofilms.²¹ The antibacterial activity of DMADDM in a commercial adhesive was dependent on the incubation period.¹² DMADDM at 2.5 wt.% was only effective in reducing bacterial viability after a longer incubation period (48 h and 72 h). At 5 wt.%, the reduction of bacterial activity was effective even after a 16-hour incubation period. Even after this longer incubation period, bacterial activity in the 2.5 wt.% group was lower than in the 5 wt.% group.¹²

When DMAHDM was tested at 0.75 wt.%, 1.5 wt.%, 2.25 wt.%, and 3 wt.% in two incubation periods (48 h and 72 h), the metabolic activity of biofilm decreased at all concentrations when compared to the control group.¹³ The higher the concentration, the greater the antibacterial activity.¹¹ Currently, better antibacterial activity and maintenance of physicochemical properties have been observed when DMAHDM is added at 5 wt.% to resin materials, such as resin composites.¹⁷

In this context, the longer the carbon chain and the concentration of QAMs, the higher the antimicrobial activity. However, these two factors can also impair resin-based physical properties.^{19,20} Besides, chemical properties such as the DC of parental resin-based materials should be considered. Efficient polymerization is a critical parameter to ensure optimal physical properties and reduce susceptibility to cytotoxic effects.²⁷ Although DMADDM and DMAHDM have been analyzed in dental materials, few studies have directly compared them.^{10,19,20} Both QAMs are very similar, with one methacrylate functional group and one ammonium functional group, with a difference of four carbons in the aliphatic chain. This study aimed to evaluate the DC, cytotoxic effects, and antibacterial activity of two QAMs (DMADDM

and DMAHDM) at three different concentrations (1, 2.5, or 5 wt.%). The null hypothesis to be tested is that the addition of QAMs does not affect the DC, cytotoxicity, and antibacterial activity of orthodontic adhesives.

Methodology

Experimental adhesive formulation

The adhesive formulation^{28,29} was the mixture of bisphenol-A-glycidyl dimetacrylate (Bis-GMA, Esstech, Essington, USA) and 2-hydroxyethyl methacrylate (HEMA, Esstech, Essington, USA) at a 50:40 wt.% ratio. As the photoinitiator/co-initiator system, 0.5 mol% of camphorquinone (Esstech Inc., Essington, USA) and 1 mol% of EDMAB (Sigma-Aldrich, St Louis, USA) were added to this base resin. QAMs were synthesized according to previous studies.^{10,15,19,30} The chemical structures of both QAMs are indicated in Figure 1 together with an illustration of the study design. DMADDM and DMAHDM were added at 1, 2.5, and 5 wt.% from the total organic matrix weight. The groups were categorized as follows:

- Transbond – commercially available Transbond XT Primer adhesive (3M Unitek, Monrovia, USA);
- Control – experimental adhesive without QAMs;
- 1% DMADDM – experimental adhesive with 1 wt.% of DMADDM;
- 2.5% DMADDM – experimental adhesive with 2.5 wt.% of DMADDM;
- 5% DMADDM – experimental adhesive with 5 wt.% of DMADDM;
- 1% DMAHDM – experimental adhesive with 1 wt.% of DMAHDM;
- 2.5% DMAHDM – experimental adhesive with 2.5 wt.% of DMAHDM;
- 5% DMAHDM – experimental adhesive with 5 wt.% of DMAHDM.

Degree of conversion

The samples of adhesives ($n = 3^{31}$) were placed on the attenuated total reflectance device of a Fourier-transform infrared (FTIR) spectrometer (Alpha-P; Bruker Optics, Ettlingen, Germany). Each sample was photoactivated for 40 s using a light-emitting diode (LED, Radii Cal, SDI, Bayswater, Victoria, Australia) with 1,200 mW/cm². This

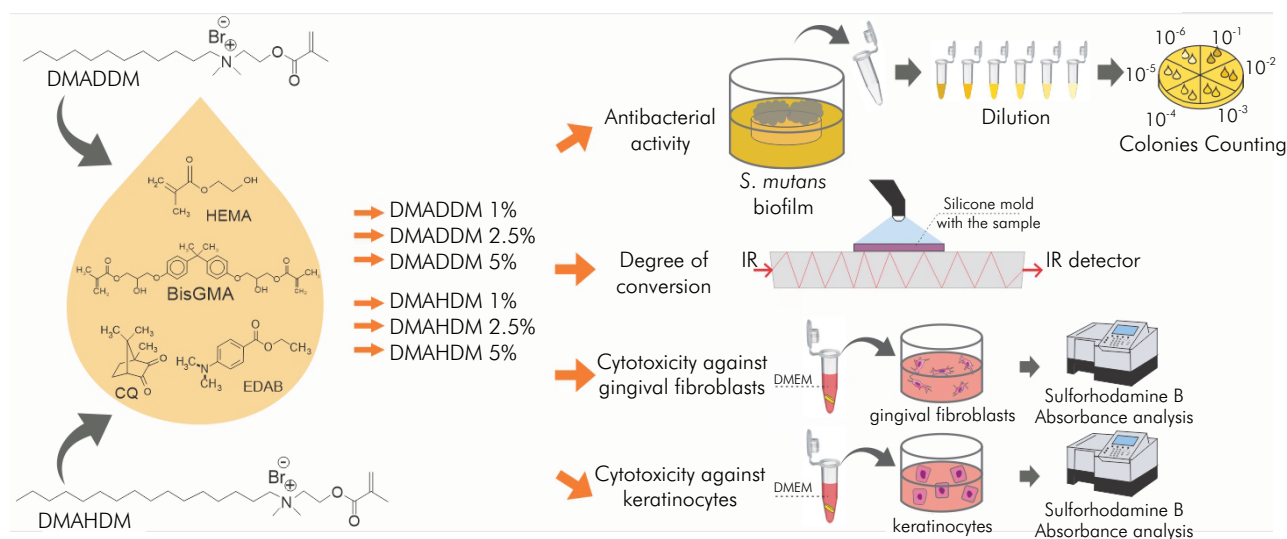


Figure 1. Illustration of the study design. The chemical structures of DMADDM and DMAHDM are indicated, evidencing longer alkyl chain (16 carbons) of DMAHDM as compared to DMADDM (12 carbons) and the presence of the quaternary ammonium group in both structures. The base resin was formulated with BisGMA, HEMA, and photoinitiators. DMADDM and DMAHDM were incorporated at 1, 2.5, and 5 wt.% into this experimental resin. All materials were tested for antibacterial activity, degree of conversion, cytotoxicity against gingival fibroblasts, and cytotoxicity against keratinocytes.

light-curing unit was used to photoactivate the adhesives throughout the study. Photoactivation was standardized at 1 mm between the tip of the light-curing unit and the top of the samples. Spectra from FTIR analysis were recorded before and immediately after the photoactivation of each sample with 32 scans and a resolution of 4 cm⁻¹. The DC was calculated based on the height of the peak at 1,638 cm⁻¹ (carbon-carbon double bonds of aliphatic chain) and the height of the peak at 1,608 cm⁻¹ peak (carbon-carbon double bonds of aromatic chain) using the following formula:

$$DC(\%) = 100 \times \frac{\frac{\text{peak height of cured aliphatic C} = \text{C}}{(\text{peak height of cured aromatic C} = \text{C})}}{\frac{\text{peak height of uncured aliphatic C} = \text{C}}{(\text{peak height of uncured aromatic C} = \text{C})}}$$

Cytotoxicity against keratinocytes

For cytotoxicity evaluation of experimental orthodontic adhesives against keratinocytes, disc-shaped samples (n = 5, 1.0 mm thickness × 4.0 mm diameter^{32,33}) were prepared after 20 s of photoactivation on each side. The samples were stored in distilled water at 37°C for 24 h, dried with absorbent paper, and sterilized using hydrogen peroxide plasma at 58 % for 48 min at 56°C. The samples were stored in 1 mL of Dulbecco's Modified Eagle Medium (DMEM, Sigma-Aldrich Chemical, St. Louis, USA) for 24 h at 37°C for eluate preparation. Human keratinocytes (HaCaT, CLS Cell Lines Service GmbH, Eppelheim, Germany) were placed at 5 × 10³ per well in 96-well plates to be treated with 100 µL of eluate from each sample. After 72 h of incubation at 37°C with 5% CO₂, cells were fixed on the bottom of the wells by trichloroacetic acid solution at 10 vol.%. The plates were incubated for 1 h at 4°C, washed with running water for 30 s, and dried at room temperature. The cells were stained with an SRB sodium salt (SRB, Sigma-Aldrich Chemical) at 0.4 vol.%. After 30 min at room temperature, the plates were washed with acetic acid 1 vol.% and dried at room temperature. Trizma solution (10 mM, 100 µL) was added to each well to dissolve the dye before absorbance assessment. After 1 h, absorbance was

analyzed at 560 nm. A negative control composed of cells without contact with eluates was used as 100%. The results were expressed in percentages of cell viability.

Cytotoxicity against gingival fibroblasts

Gingival fibroblasts were collected after a periodontal procedure and informed consent was obtained from the patient after approval by the local Research Ethics Committee (process n° 1.739.340). The patient donated the tissue after its removal according to therapeutic indication after signing the informed consent form. Firstly, the cells were grown in flasks containing nutrient medium. The medium was composed of DMEM with 10% fetal bovine serum, 5 mM of Hepes, 3.7 g sodium bicarbonate, 100 U/mL of penicillin, and 100 mg/mL of streptomycin. The cells were incubated at 37°C with 5% CO₂. The cells were evaluated daily with the aid of an inverted-phase microscope, and the medium was changed every 2 to 3 days. After cell growth, new samples of adhesives were prepared (n = 5, 1.0 mm thickness × 4.0 mm diameter^{32,33}) with photoactivation for 20 s on each side. The samples were stored in distilled water at 37°C for 24 h, dried with absorbent paper, and sterilized using hydrogen peroxide plasma at 58 % for 48 min at 56°C. The samples used to produce the eluates were prepared according to the description above for the evaluation of cytotoxicity against keratinocytes. The cells were seeded (5 × 10³ per well) in 96-well plates and treated with 100 µL of eluate from each sample. The contact period between the eluates and the cells was 72 h. Thereafter, the cells were treated according to the description above using the SRB method. The absorbance results were also normalized against the cell viability in wells with cells without contact with the eluates and expressed as percentage.

Antibacterial activity against biofilm formation

To evaluate antibacterial activity, disc-shaped samples of the orthodontic adhesives were prepared (n = 3, 1.0 mm thickness × 4.0 mm diameter^{16,34}) with photoactivation for 20 s on each side. The samples were stored in distilled water at 37°C for

24 h and dried with absorbent paper. The samples were attached onto the lid of a 48-well plate, and this set was sterilized using hydrogen peroxide plasma at 58 % for 48 min at 56°C. In each well of the 48-well plate, 900 µL of brain heart infusion (BHI) broth was added. The BHI broth contained 1 wt.% of sucrose and 100 µL of a suspension of an overnight broth culture of *S. mutans* (NCTC 10449) at 10⁶ CFU/mL. The 48-well plate containing the samples was incubated for 24 h at 37°C. After that, the samples were detached from the lid, vortexed for 1 min in 1 mL of sterile saline solution (0.9 wt.%) in an Eppendorf tube. The solution was serially diluted up to 10⁻⁶ mL of saline solution. Then, two drops of 25 µL were plated on Petri dishes with BHI agar. The plates were incubated for 48 h at 37°C.^{16,34}

Statistical analysis

The data were analyzed using SigmaPlot software (version 12.0, Systat Software, San Jose, USA). Data distribution was evaluated by the Shapiro-Wilk test. DC, cytotoxicity against fibroblasts, and antibacterial activity were analyzed using one-way ANOVA and Tukey's multiple comparisons. Cytotoxicity against keratinocytes was evaluated using the Kruskal Wallis

and Dunn's post-hoc tests. A significance level of 5% was used for all tests.

Results

The DC of the adhesives is displayed in Figure 2. Transbond showed lower DC than 5% DMAHDM, 1% DMADDM, and 5% DMADDM ($p < 0.05$). There were no statistically significant differences among the other groups ($p > 0.05$).

The results for keratinocyte viability are presented in Table 1. There were statistically significant differences among the experimental adhesive groups ($p < 0.05$). Transbond showed no difference for the control group, neither for the adhesives containing 1% or 5% of QAMs ($p > 0.05$). There was a statistically significant difference between Transbond and both groups with 2.5 wt.% of QAMs ($p < 0.05$), without statistical difference between 2.5% DMAHDM and 2.5% DMAHDM ($p > 0.05$).

The results for fibroblast viability are displayed in Figure 3. The addition of 5 wt.% of DMAHDM reduced cell viability when compared to the control group and Transbond ($p < 0.05$). The group with 5 wt.% of DMADDM showed lower cell viability than

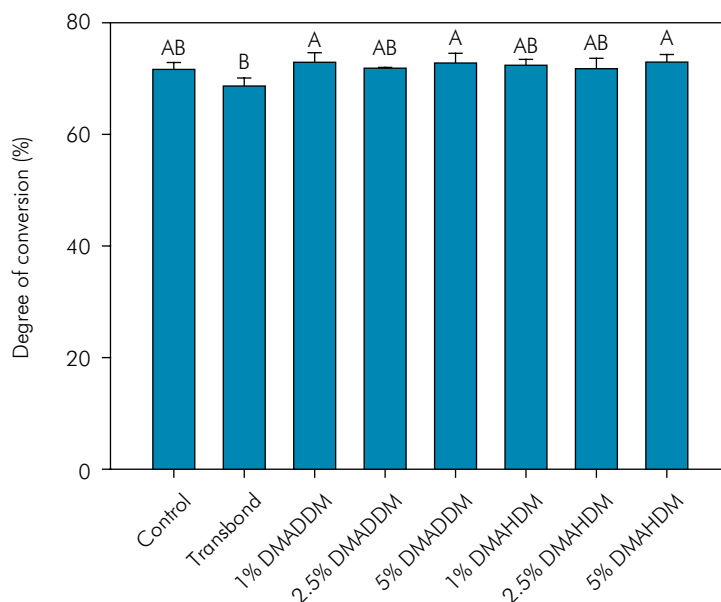


Figure 2. Mean and standard deviation of degree of conversion (%) of the orthodontic adhesives. Different capital letters indicate statistically significant differences among groups ($p > 0.05$).

Table 1. Keratinocyte viability after contact with eluates from polymerized samples.

Group	Median (50th percentile)	25th percentile	75th percentile	Statistical result
Control	23.20	19.48	26.84	AB
Transbond	98.72	96.78	99.44	A
1% DMADDM	17.53	15.29	29.93	AB
2.5% DMADDM	13.03	10.30	16.51	B
5% DMADDM	23.50	16.21	26.20	AB
1% DMAHDM	15.72	14.01	30.13	AB
2.5% DMAHDM	12.61	11.76	13.82	B
5% DMAHDM	15.42	14.67	20.81	AB

Different capital letters indicate a statistically significant difference among groups ($p < 0.05$).

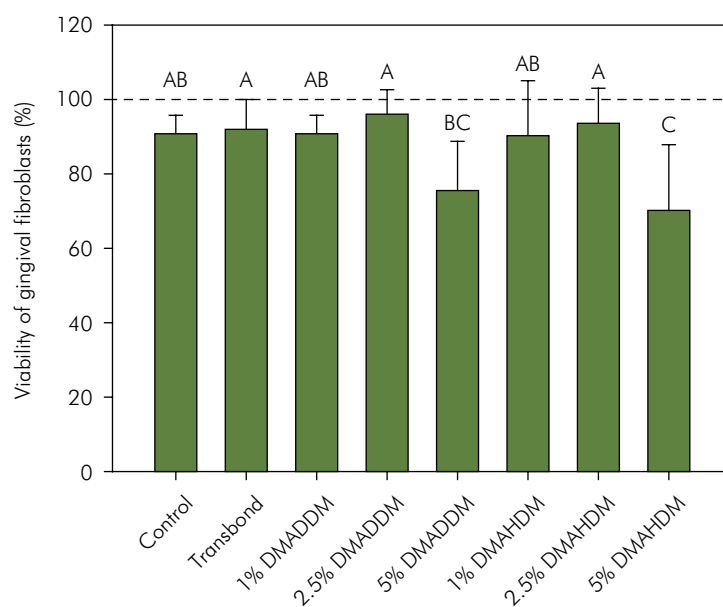


Figure 3. Mean and standard deviation of gingival fibroblast viability (%). Different capital letters indicate statistically significant differences among groups ($p < 0.05$).

Transbond ($p < 0.05$), without statistical difference for the control group ($p > 0.05$). There was no difference between the groups containing 5 wt.% of QAMs ($p > 0.05$).

Figure 4 indicates the log CFU/mL of viable *S. mutans* on biofilm formed on the polymerized samples. The group with 5 wt.% of DMAHDM induced more than 1 log reduction in relation to the control and Transbond groups ($p < 0.05$). There was a statistical difference between the groups, with lower bacterial viability for the group with 5 wt.% of DMAHDM as compared to all the other adhesives ($p < 0.05$), without

statistically significant difference among the other groups ($p > 0.05$).

Discussion

In the present study, two types of QAMs were explored in orthodontic adhesives at different concentrations. The formulated materials, together with a commercial control (Transbond), were analyzed for chemical and biological properties. There was no difference in DC among the groups. However, biological properties differed among groups in

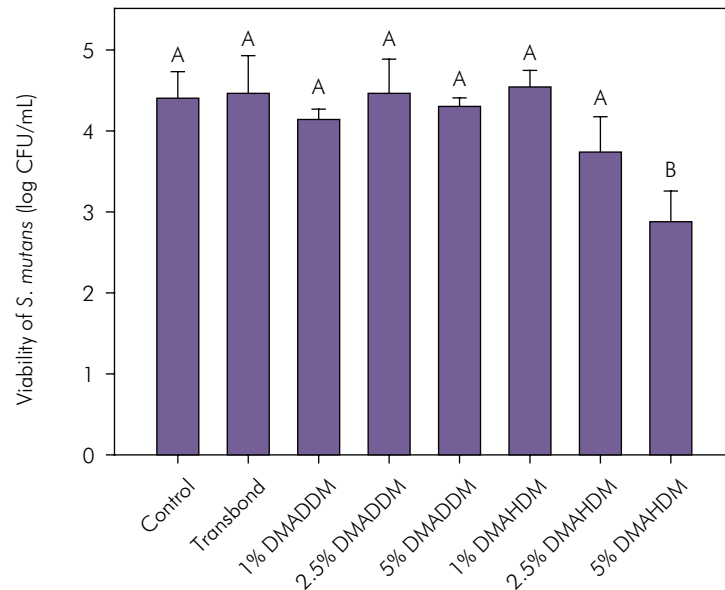


Figure 4. Mean and standard deviation of antibacterial activity against biofilm formation after contact with the orthodontic adhesives. Different capital letters indicate statistically significant differences among groups ($p < 0.05$).

cytotoxicity and antibacterial activity evaluations. Therefore, the null hypothesis was partially rejected.

High DC is essential for achieving proper mechanical properties and hydrolytic stability of dental adhesives.³¹ The incorporation of monomers with different densities and quantity of methacrylate groups affects the polymerization kinetics and the DC of adhesives.³¹ In this way, the DC of resins doped with QAMs should be analyzed to help elucidate the polymerization behavior of the developed materials. Transbond showed lower DC than the groups with 5 wt.% of QAMs and 1% DMADDM ($p < 0.05$). It was not clear why there was statistically significant difference among these four groups. However, we could observe that, regardless of the statistical outcome, all groups showed proper DC when compared to the literature, achieving more than 50 % of DC.^{35,36}

Considering ISO 10993-5, all experimental adhesives showed cytotoxicity against keratinocytes because all these groups presented values above 70%.³⁷ Previous studies have reported higher viability when DMAHDM at 5 wt.% was used in sealants²³ and DMADDM at 2.5 and 5 wt.% was used in adhesive resins.¹² By contrast, another study that tested solutions containing DMADDM showed

significant cytotoxic effects on human buccal epithelial cells.³⁸ All experimental adhesives showed cell viability lower than 70%, while Transbond presented higher values, suggesting that other components and concentrations could be involved in this process and lead to cytotoxicity.

The cytotoxicity of monomers was ranked as follows: BisGMA > UDMA > TEGDMA > HEMA.³⁹ Transbond is composed of TEGDMA, BisGMA, and photoinitiator systems. Therefore, the eluted HEMA from our adhesives may have affected the viability of keratinocytes. Moreover, differences in network formation between experimental adhesives and Transbond could differ in quantity and type of components release. The risk of adverse effects on other tissues is emphasized because these materials can release a great variety of toxic compounds, which may diffuse across biological membranes and, therefore, exert adverse effects either locally or beyond the oral cavity.⁴⁰ Although BisGMA has been identified as one of the most cytotoxic monomers,^{39,40} it is widely used in dental resins. DMADDM, which is 20 times less toxic than BisGMA, could probably also be acceptable for clinical use.⁴¹

Cytotoxicity tests are performed to determine how a specific cell type could be affected by an

experimental material. Of note, the *in vitro* test applied may have overestimated the cytotoxic effect of the materials. In bracket bonding or cavity restoration, only a thin layer of adhesive is used, which contrasts with the thickness of the samples and with the storage conditions used. However, this strategy is employed to observe the behavior of materials when subjected to major challenges, using a high concentration of eluates (the eluates were not diluted) and high exposure (72 hours). Although the experiments with *in vitro* cells cannot reproduce *in vivo* conditions, they are widely used as a simplified method of investigation and restrict the number of experimental variables.³⁷

The evaluation of cytotoxicity against fibroblasts indicated a higher percentage of cell viability. All experimental groups showed values over 70%, which is in line with previous analyses using fibroblasts.⁴² We decided to test the possible cytotoxicity of the adhesives against fibroblasts because of the original site of these cells (gingiva) and its possible proximity to the orthodontic adhesive in a clinical situation. Furthermore, ISO recommends the use of fibroblasts to analyze the cytotoxicity potential of materials.³⁷ The findings reported herein for fibroblast viability suggest that the adhesives with DMAHDM or DMADDM up to 5 wt.% do not present cytotoxicity.

The antibacterial activity was assessed via quantification of viable *S. mutans* on the biofilm formed on the polymerized samples. The group containing DMAHDM at 5 wt.% showed antibacterial activity with a reduction of more than 1 log CFU/mL compared to the control groups. A previous study found that an increase in DMADDM mass fraction reduces the metabolic activity of biofilms.⁴³ However, in the present study, bacterial viability did not change for DMADDM groups after diluting and plating the bacterial content. This difference may be associated with the antibacterial tests applied. The MTT assay is a colorimetric test to assess the metabolic activity of cells. Despite being widely used, it has been suggested that tests that analyze cell viability rather than cell metabolism be applied. As with tests based solely on morphological changes of cell membranes, tests based on metabolism generally have less predictive power.⁴⁴ For this reason, we chose to perform tests that quantify cell viability both in the analysis

of cytotoxicity (SRB method) and in the study of antibacterial activity (colony-forming units assay).

The higher antibacterial effect of DMAHDM as compared to DMADDM at the same concentration (5 wt.%) is attributed to the longer alkyl chain of DMAHDM. As mentioned earlier, DMAHDM has 16 carbons in the aliphatic chain, while DMADDM has 12. A previous study has analyzed the effect of QAMs with three, six, nine, twelve, sixteen, and eighteen carbons in the aliphatic chain against *S. mutans* biofilm. The authors observed a decreased minimum inhibitory concentration (MIC) by increasing the aliphatic chain of QAM. Moreover, there was decreased biofilm formation on polymerized samples with longer-chain methacrylates.²⁵ Another study evaluated this effect against microcosm biofilm derived from dental plaque. From three to sixteen carbons, there was also improved antibacterial property.²⁵ The rationale for these outcomes is the higher hydrophobicity and improved capacity of DMAHDM to penetrate the hydrophobic microbial membrane compared to QAMs with shorter aliphatic chains.²⁵

As a limitation of this study, we did not quantify the released compounds. Further research could analyze this effect after soaking the materials in solutions for different periods. This analysis could contribute to a deeper understanding of the biological effects of materials. The formulated orthodontic adhesives present a comonomer blend that could also be used for dentin bonding. Therefore, further research could also be performed by analyzing adhesives with 5 wt.% of DMAHDM to improve antibacterial activity and assist in preventing recurrent caries around resin-based restorations.²

Conclusion

The addition of QAMs did not change DC when compared to the experimental control group, and some QAM groups had higher DC than Transbond. Even though there was decreased keratinocyte viability, gingival fibroblasts showed proper viability. Furthermore, DMAHDM at 5 wt.% decreased *Streptococcus mutans* viability. The incorporation of DMAHDM at 5 wt.% may be a strategy for reducing the development of WSLs.

References

1. Cheng L, Weir MD, Zhang K, Arola DD, Zhou X, Xu HH. Dental primer and adhesive containing a new antibacterial quaternary ammonium monomer dimethylaminododecyl methacrylate. *J Dent.* 2013 Apr;41(4):345-55. <https://doi.org/10.1016/j.jdent.2013.01.004>
2. Degrazia FW, Leitune VC, Garcia IM, Arthur RA, Samuel SM, Collares FM. Effect of silver nanoparticles on the physicochemical and antimicrobial properties of an orthodontic adhesive. *J Appl Oral Sci.* 2016;24(4):404-10. <https://doi.org/10.1590/1678-775720160154>
3. Melo MA, Wu J, Weir MD, Xu HH. Novel antibacterial orthodontic cement containing quaternary ammonium monomer dimethylaminododecyl methacrylate. *J Dent.* 2014 Sep;42(9):1193-201. <https://doi.org/10.1016/j.jdent.2014.07.006>
4. 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 Dec;43(12):1539-46. <https://doi.org/10.1016/j.jdent.2015.09.004>
5. Buschang PH, Chastain D, Keylor CL, Crosby D, Julien KC. Incidence of white spot lesions among patients treated with clear aligners and traditional braces. *Angle Orthod.* 2019 May;89(3):359-64. <https://doi.org/10.2319/073118-553.1>
6. Armstrong D, Shen G, Petocz P, Darendeliler MA. Excess adhesive flash upon bracket placement. A typodont study comparing APC PLUS and Transbond XT. *Angle Orthod.* 2007 Nov;77(6):1101-8. <https://doi.org/10.2319/081506-334.1>
7. Altmann AS, Degrazia FW, Celeste RK, Leitune VC, Samuel SM, Collares FM. Orthodontic bracket bonding without previous adhesive priming: a meta-regression analysis. *Angle Orthod.* 2016 May;86(3):391-8. <https://doi.org/10.2319/041615-255.1>
8. Akhavan A, Sodagar A, Mojtahedzadeh F, Sodagar K. Investigating the effect of incorporating nanosilver/nanohydroxyapatite particles on the shear bond strength of orthodontic adhesives. *Acta Odontol Scand.* 2013 Sep;71(5):1038-42. <https://doi.org/10.3109/00016357.2012.741699>
9. Melo MA, Orrego S, Weir MD, Xu HH, Arola DD. Designing multiagent dental materials for enhanced resistance to biofilm damage at the bonded interface. *ACS Appl Mater Interfaces.* 2016 May;8(18):11779-87. <https://doi.org/10.1021/acsami.6b01923>
10. Assad-Loss TF, Vignoli JF, Garcia IM, Portela MB, Schneider LF, Collares FM, et al. Physicochemical properties and biological effects of quaternary ammonium methacrylates in an experimental adhesive resin for bonding orthodontic brackets. *J Appl Oral Sci.* 2021 May;29:e20201031. <https://doi.org/10.1590/1678-7757-2020-1031>
11. Feng X, Zhang N, Xu HH, Weir MD, Melo MA, Bai Y, et al. Novel orthodontic cement containing dimethylaminohexadecyl methacrylate with strong antibacterial capability. *Dent Mater J.* 2017 Sep;36(5):669-76. <https://doi.org/10.4012/dmj.2016-370>
12. Liang J, Li M, Ren B, Wu T, Xu HH, Liu Y, et al. The anti-caries effects of dental adhesive resin influenced by the position of functional groups in quaternary ammonium monomers. *Dent Mater.* 2018 Mar;34(3):400-11. <https://doi.org/10.1016/j.dental.2017.11.021>
13. Wang H, Wang S, Cheng L, Jiang Y, Melo MA, Weir MD, et al. Novel dental composite with capability to suppress cariogenic species and promote non-cariogenic species in oral biofilms. *Mater Sci Eng C.* 2019 Jan;94:587-96. <https://doi.org/10.1016/j.msec.2018.10.004>
14. 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 Sep;31(9):1119-31. <https://doi.org/10.1016/j.dental.2015.06.010>
15. 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 May;28(5):561-72. <https://doi.org/10.1016/j.dental.2012.01.005>
16. Garcia IM, Souza VS, Hellriegel C, Scholten JD, Collares FM. Ionic liquid-stabilized titania quantum dots applied in adhesive resin. *J Dent Res.* 2019 Jun;98(6):682-8. <https://doi.org/10.1177/0022034519835203>
17. Balhaddad AA, Garcia IM, Mokeem L, Ibrahim MS, Collares FM, Weir MD, et al. Bifunctional composites for biofilms modulation on cervical restorations. *J Dent Res.* 2021 Sep;100(10):1063-71. <https://doi.org/10.1177/00220345211018189>
18. Li Y, Hu X, Ruan J, Arola DD, Ji C, Weir MD, et al. Bonding durability, antibacterial activity and biofilm pH of novel adhesive containing antibacterial monomer and nanoparticles of amorphous calcium phosphate. *J Dent.* 2019 Feb;81:91-101. <https://doi.org/10.1016/j.jdent.2018.12.013>
19. Rego GF, Vidal ML, Viana GM, Cabral LM, Schneider LF, Portela MB, et al. Antibiofilm properties of model composites containing quaternary ammonium methacrylates after surface texture modification. *Dent Mater.* 2017 Oct;33(10):1149-56. <https://doi.org/10.1016/j.dental.2017.07.010>
20. Vidal ML, Rego GF, Viana GM, Cabral LM, Souza JP, Silikas N, et al. Physical and chemical properties of model composites containing quaternary ammonium methacrylates. *Dent Mater.* 2018 Jan;34(1):143-51. <https://doi.org/10.1016/j.dental.2017.09.020>
21. Wang S, Zhang K, Zhou X, Xu N, Xu HH, Weir MD, et al. Antibacterial effect of dental adhesive containing dimethylaminododecyl methacrylate on the development of *Streptococcus mutans* biofilm. *Int J Mol Sci.* 2014 Jul;15(7):12791-806. <https://doi.org/10.3390/ijms150712791>
22. 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 Apr;30(4):433-41. <https://doi.org/10.1016/j.dental.2014.01.002>

23. Ibrahim MS, Balhaddad AA, Garcia IM, Hefni E, Collares FM, Martinho FC, et al. Tooth sealing formulation with bacteria-killing surface and on-demand ion release/recharge inhibits early childhood caries key pathogens. *J Biomed Mater Res B Appl Biomater*. 2020 Nov;108(8):3217-27. <https://doi.org/10.1002/jbm.b.34659>
24. Nohr RS, Macdonald JG. New biomaterials through surface segregation phenomenon: new quaternary ammonium compounds as antibacterial agents. *J Biomater Sci Polym Ed*. 1994;5(6):607-19. <https://doi.org/10.1163/156856294X00239>
25. Li F, Weir MD, Xu HH. Effects of quaternary ammonium chain length on antibacterial bonding agents. *J Dent Res*. 2013 Oct;92(10):932-8. <https://doi.org/10.1177/0022034513502053>
26. van Tonder A, Joubert AM, Cromarty AD. Limitations of the 3-(4,5-dimethylthiazol-2-yl)-2,5-diphenyl-2H-tetrazolium bromide (MTT) assay when compared to three commonly used cell enumeration assays. *BMC Res Notes*. 2015 Feb;8(1):47. <https://doi.org/10.1186/s13104-015-1000-8>
27. Franz A, König F, Lucas T, Watts DC, Schedle A. Cytotoxic effects of dental bonding substances as a function of degree of conversion. *Dent Mater*. 2009 Feb;25(2):232-9. <https://doi.org/10.1016/j.dental.2008.07.003>
28. Park J, Ye Q, Topp EM, Misra A, Kieweg SL, Spencer P. Effect of photoinitiator system and water content on dynamic mechanical properties of a light-cured bisGMA/HEMA dental resin. *J Biomed Mater Res A*. 2010 Jun;93(4):1245-51.
29. Wang Y, Spencer P, Yao X, Ye Q. Effect of coinitiator and water on the photoreactivity and photopolymerization of HEMA/camphorquinone-based reactant mixtures. *J Biomed Mater Res A*. 2006 Sep;78(4):721-8. <https://doi.org/10.1002/jbm.a.30733>
30. 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 Feb;28(2):219-28. <https://doi.org/10.1016/j.dental.2011.10.004>
31. Collares FM, Portella FF, Leitune VC, Samuel SM. Discrepancies in degree of conversion measurements by FTIR. *Braz Oral Res*. 2013;27(6):453-4.
32. Garcia IM, Leitune VC, Arthur RA, Nunes J, Visioli F, Giovarruscio M, et al. Chemical, Mechanical and Biological Properties of an Adhesive Resin with Alkyl Trimethyl Ammonium Bromide-loaded Halloysite Nanotubes. *J Adhes Dent*. 2020;22(4):399-407.
33. Mena Silva PA, Garcia IM, Nunes J, Visioli F, Castelo Branco Leitune V, Melo MA, et al. Myristyltrimethylammonium Bromide (MYTAB) as a cationic surface agent to inhibit *Streptococcus mutans* grown over dental resins: an *in vitro* study. *J Funct Biomater*. 2020 Feb;11(1):9. <https://doi.org/10.3390/jfb11010009>
34. Martini Garcia I, Jung Ferreira C, de Souza VS, Castelo Branco Leitune V, Samuel SM, de Souza Balbinot G, et al. Ionic liquid as antibacterial agent for an experimental orthodontic adhesive. *Dent Mater*. 2019 Aug;35(8):1155-65. <https://doi.org/10.1016/j.dental.2019.05.010>
35. Collares FM, Ogliairi FA, Zanchi CH, Petzhold CL, Piva E, Samuel SM. Influence of 2-hydroxyethyl methacrylate concentration on polymer network of adhesive resin. *J Adhes Dent*. 2011 Apr;13(2):125-9.
36. Gaglianone LA, Lima AF, Gonçalves LS, Cavalcanti AN, Aguiar FH, Marchi GM. Mechanical properties and degree of conversion of etch-and-rinse and self-etch adhesive systems cured by a quartz tungsten halogen lamp and a light-emitting diode. *J Mech Behav Biomed Mater*. 2012 Aug;12:139-43. <https://doi.org/10.1016/j.jmbbm.2012.01.018>
37. International Organization for Standardization. ISO 10993-5. Biological evaluation of medical devices. Part 5: Tests for cytotoxicity: *in vitro* methods. 1999;1-60. Geneva: International Organization for Standardization; 2009.
38. Zhou Y, Wang S, Zhou X, Zou Y, Li M, Peng X, et al. Short-time antibacterial effects of dimethylaminododecyl methacrylate on oral multispecies biofilm *in vitro*. *BioMed Res Int*. 2019 Jan;2019:6393470. <https://doi.org/10.1155/2019/6393470>
39. Ratanasathien S, Wataha JC, Hanks CT, Dennison JB. Cytotoxic interactive effects of dentin bonding components on mouse fibroblasts. *J Dent Res*. 1995 Sep;74(9):1602-6. <https://doi.org/10.1177/00220345950740091601>
40. Bandarra S, Mascarenhas P, Luís AR, Catrau M, Bekman E, Ribeiro AC, et al. *In vitro* and *in silico* evaluations of resin-based dental restorative material toxicity. *Clin Oral Investig*. 2020 Aug;24(8):2691-700. <https://doi.org/10.1007/s00784-019-03131-4>
41. Li F, Weir MD, Fouad AF, Xu HH. Time-kill behaviour against eight bacterial species and cytotoxicity of antibacterial monomers. *J Dent*. 2013 Oct;41(10):881-91. <https://doi.org/10.1016/j.jdent.2013.07.006>
42. 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 Apr;29(4):450-61. <https://doi.org/10.1016/j.dental.2013.01.012>
43. 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 Aug;29(8):859-70. <https://doi.org/10.1016/j.dental.2013.05.005>
44. Kreth J, Ferracane JL, Pfeifer CS, Khajotia S, Merritt J. At the interface of materials and microbiology: a call for the development of standardized approaches to assay biomaterial-biofilm interactions. *J Dent Res*. 2019 Jul;98(8):850-2. <https://doi.org/10.1177/0022034519854685>