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Review Article

A comprehensive review of the antibacterial activity of dimethylaminohexadecyl methacrylate (DMAHDM) and its influence on mechanical properties of resin-based dental materials

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ABSTRACT

The repetitive restorative cycle should be avoided, aiming at the smallest number of restorations' replacements to ensure greater tooth longevity. Antibacterial materials associated with the control of caries etiological factors can help improve restoration's durability. This review aimed to analyze the results of *in vitro* studies that added Dimethylaminohexadecyl methacrylate (DMAHDM), an antibacterial monomer, to restorative materials. The PubMed, SCOPUS, Web of Science, and Biblioteca Virtual em Saúde databases were screened for studies published between 2015 and 2020. After full-text reading, 24 articles were included in the final sample. DMAHDM has demonstrated antibacterial efficacy against several bacteria related to dental caries and periodontal diseases, causing a transition in the biofilm balance without inducing resistance. When DMAHDM was included in acrylic resin, the material cytotoxicity increased, and changes in mechanical properties were observed. In contrast, resin composites had their mechanical properties maintained in most studies; however, toxicity was not examined. The association between DMAHDM and 2-methacryloyloxyethyl phosphorylcholine or silver nanoparticles improved the antibacterial effect. Besides, the association with nanoparticles of amorphous calcium phosphate or nanoparticles of calcium fluoride can provide remineralization capacity. There is a lack of information on the cytotoxicity and bacteria resistance induction, and further studies are needed to address this.

1. Introduction

Dental caries is one of the most common bacterial infections in the human body. It represents a global public health problem with serious economic consequences, affecting an estimated 2.3 billion of the world's population [1,2]. The disease's underlying mechanism consists of demineralization caused by the acid attack of bacteria present in the dental biofilm [3–5]. The growth of such acidogenic bacteria in the biofilm is possible from a diet rich in fermentable carbohydrates and poor oral hygiene [6,7].

The advance of the carious process may require the restoration of lost dental tissues so that resin composites are widely used with this aim due to their esthetics and ability to fill tooth preparations directly [8,9]. Much effort has been made to decrease polymerization contraction stresses and improve surface polishing to provide greater restoration longevity. However, factors, such as restoration's surface location, patient's caries susceptibility, socioeconomic status or age, operator's skill and the criteria for lesion detection, can still contribute to the occurrence of recurrent caries [10–12].

Several studies report the frequent presence of caries lesions at the restorations' margins, so that a high occurrence of restoration replacement is observed [8,13–17]. From this perspective, a composite resin with antibacterial properties could help in this process and reduce caries lesions at the restoration margins by interfering with the restorations' adjacent dental biofilm. An antimicrobial composite resin, associated with the control of caries' etiological factors, would be of great value in this process.

In this context, the association of resin composites with quaternary ammonium monomers (QAM) would inhibit biofilm growth due to this monomer's capacity to cause bacterial lysis by breaking

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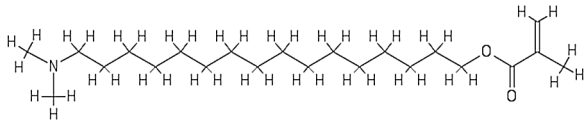

Fig. 1. Chemical structure of the DMAHDM monomer.

the cellular membrane [18]. QAM's antimicrobial activity's exact mechanism consists of its binding to bacterial cell membranes, causing cytoplasmic leaks by disturbing the electrical balance and consequent bacterial lysis due to osmotic pressure. This process is made possible by encountering the negative bacterial cell and positive charge sites in the QAM, causing death by contact [19,20].

Several studies have evaluated the antibacterial effect of dimethylaminohexadecyl methacrylate (DMAHDM) (Fig. 1), a recently synthesized QAM [21–25]. Thus, this paper aims to discuss the role of DMAHDM in resin-based dental materials concerning its immediate and long-term antibacterial activity, cytotoxicity, and how it interacts with other chemical components in dental resins. A bibliographic search was performed on PubMed, Web of Science, SCOPUS, and Biblioteca Virtual em Saúde (BVS) research databases, using only "DMAHDM" as a descriptor due to the scarcity of studies. We considered papers published from 2015 to 2020, and all types of study designs were included. The results of our research can be found in Table 1.

2. Mechanism of action

Quaternary ammonium monomers have mechanisms of action similar to quaternary ammonium salts (QAS). Long-chain quaternary ammonium compounds can penetrate the bacterial cell membrane, disturbing the lipid layers' balance, causing the leaking of the bacterial cytoplasmic content [26,27]. Another possible mechanism occurs from the contact between the positively charged N+ quaternary amine in the structure of the QAS with the negatively charged bacterial membrane. This contact causes the loss in the balance of essential ions, disturbance in the membrane functions, such as respiration, transport of solutes, and biosynthesis of the cellular wall [22,28] be seen in Fig. 2.

It is also suggested that quaternary ammonium surfactant, a sub-group of the quaternary ammonium compounds, may alter the superficial electrostatic charge in contact with the bacterial cell membrane. The cell membrane would become more positive, causing the collapse of the proton-motive force (PMF), an electrochemical gradient of protons critical for ATP production [29,30]. Thus, it is possible to suggest that the loss of the capacity of multiplication of bacterial DNA and protein activity in the cell pointed out in previous studies is associated with the collapse of the PMF and consequent loss of the production of ATP, considering that protein synthesis and replication of DNA require ATP [22,31–33].

3. Antibacterial properties

As highlighted above, from contact between the negatively charged bacterial cell wall and the positive charge of the quaternary amine present in the monomer, it becomes possible to disturb the cell wall's electrical balance, leading to bacterial death [34]. This mechanism allowed a good bactericidal efficacy to be achieved in several resin compositions, bringing benefits that can be further enhanced by the association with different components.

Regarding the antibacterial potential of DMAHDM monomer, all studies included in this review used several tests to verify this property: Colony Formation Units (CFU), live/dead biofilm staining assay, the phenol-sulfuric acid method to evaluate polysaccharide production, enzymatic (lactate dehydrogenase) method to measure lactic acid production, and MTT (3-[4,5-dimethylthiazol-2-yl]-2,5-diphenyltetrazolium bromide) assay of metabolic activity of biofilms. The comparison between studies should be made with caution since there is great heterogeneity due to variations in the use of DMAHDM alone or associated with other components, tests of different concentrations, and biofilms of various species. Firstly, it is remarkable in all 25 studies that the monomer's antibacterial efficacy has been confirmed through the adopted tests. All of them found a better performance of DMAHDM than the control.

The live/dead biofilm staining assay demonstrated that the experimental resins were mainly covered with dead bacteria [23–25,35–51], with live bacteria covering 10% to 20% of the surface of the resins, while controls ranged from 50% to 100% [46,48]. Polysaccharide production showed reduction values between 2 and 6 times higher than the control [23,36–39,42,44,47,49,51]. There was also a reduction in metabolic activity [23–25,35–39,42–48,50–53], biofilm biomass [36,41], and lactic acid production [24,25,36,38–46,50,51,53] indicated in the studies, demonstrating that the monomer can inhibit the bacterial activity responsible for the appearance of caries lesions. Another important point is the evaluation of the CFU count in experimental resins. There was a great variation in the composition of the biofilms analyzed. However, DMAHDM reduced CFU formation in all studies, ranging from a minimum of 3 to a maximum of 6 orders of magnitude [23–25,36–51,53].

The pathogenesis of caries involves lowering the local pH due to acids' production from the metabolism of fermentable carbohydrates by bacterial colonies present in dental biofilm [2]. The evidence pointed out above for the inhibition of polysaccharide production, lactic acid, bacterial metabolism, and CFU count suggests that the DMAHDM monomer's use may directly promote bacterial death, avoiding the successive events necessary for the formation of caries lesions.

In addition to reducing bacterial microbiota, DMAHDM can also alter the biofilm balance to increase the number of commensal species, such as *S. sanguinis* and *S. gordonii* [51]. Previous studies state that the commensal species mentioned are primary colonizers. They can antagonize *S. mutans*, producing hydrogen peroxide and neutralizing the acids in the biofilm [54–57]. This finding justifies a higher proportion of *S. sanguinis* and *S. gordonii* in the biofilm of healthy tooth surfaces and caries-free individuals. Conversely, a higher proportion of *S. mutans* indicates acidogenicity associated with individuals with caries [55,58,59].

Adding the evidence of inhibition of the mechanisms of caries pathogenesis to the change in biofilm balance shown above, we can strongly suggest that the DMAHDM monomer incorporated in restorative materials represents a possibility of an advantage to act in the carious process when compared to conventional resin composites. Therefore, the use of such a component can be seen as a preventive tool to avoid recurrent carious lesions, given that it would act early in the mechanism of the disease.

Still, the studies analyzed did observe not only cariogenic species but also periodontopathogenic species such as *P. gingivalis*, *P. intermedia*, *A. actinomycetemcomitans*, and *F. nucleatum*. Three studies [37,47,49] evaluated the inhibition of such species provided by the DMAHDM and found excellent results compared with the control evidenced by the reduction in UFC count, bacterial metabolism, polysaccharide production, and biofilm biomass. Thus, it is likely that DMAHDM has the potential to prevent the occurrence of dental caries and be an important tool in the prevention of gingival and periodontal diseases.

3.1. Bacterial resistance

Wang et al. (2018) [60] evaluated the development of resistance of *S. mutans*, *S. sanguinis*, and *S. gordonii* in planktonic forms and

Table 1
Data extracted from primary studies.

Authors	Material used together	Mechanical properties analyzed	Antibacterial properties
Wu et al. (2015) [25]	20% nanoparticles of amorphous calcium phosphate (NACP)	• 3.75% DMAHDM (by wt%): Flexural strength: similar values to control. Modulus of elasticity: similar values to control.	3.75% DMAHDM (by wt%): • Dead/alive bacteria: increased adherent compromised bacteria. • Metabolic activity: values between 0 and 0.2 $A_{540/}cm^2$ (control between 1 and 1.2 A_{540}/cm^2). • Lactic acid production: approximate value 0 mmol/L (control between 20 and 25 mmol/L). • CFU: reduced the CFU between 3 and 4 log. Values between 10^4 and 10^7 (control between 10^7 and 10^{10} CFU) in a biofilm of *S. mutans*, total Streptococci, and total microorganisms.
Wu et al. (2015) [45]	30% NACP	• 0.75–3% DMAHDM (by wt%): Fracture toughness: similar values to control.	3% DMAHDM (by wt%): • Dead/alive bacteria: increased adherent compromised bacteria as the percentage of DMAHDM increased from 0.75–3%. The area of live bacteria with 3% DMAHDM was close to 10% (control between 90 and 100%). • Metabolic activity: approximate value 0 $A_{540/}cm^2$ (control 1 A_{540}/cm^2). • Lactic acid production: approximate value 0 mmol/L (control 24 mmol/L). • CFU: Reduced the CFU by 3 log. Values between 10^4 and 10^8 (control between 10^7 and 10^{11}), in a biofilm of *S. mutans*, total Streptococci, and total microorganisms.
Zhang et al. (2015) [46]	3% MPC	• 3% DMAHDM (by wt%): Flexural strength: similar values to control. Modulus of elasticity: similar values to control With 3% MPC: it could only be added up to 1.5% DMAHDM without changing the mechanical properties;	1.5% DMAHDM (by wt%): • Dead/alive bacteria: increased adherent compromised bacteria. Values close to 20% coverage of the area with live bacteria (controls close to 100%).
Wang et al. (2016) [47]	3% MPC	• 3% DMAHDM (by wt%): Flexural strength: similar values to control. Modulus of elasticity: similar values to control. 4.5% DMAHDM changed the properties.	3% DMAHDM (by wt%): • Dead/alive bacteria: increased adherent compromised bacteria. • CFU: Reduced the CFU by 4 log. Values between 10^5 and 10^7 (control between 10^9 and 10^{10}) in biofilms of *P. gingivalis*, *P. intermedia*, *A. actinomycetemcomitans*, and *F. nucleatum*. • Metabolic activity: approximate value $0.2OD_{540}/cm^2$ (control between 0.8 and $0.9OD_{540/}cm^2$). • Polysaccharide production: values between 20 and 30 mg/L (control between 60 and 80 mg/L).
Wang et al. (2016) [49]	20% NACP	• 3% DMAHDM (by wt%): Flexural strength: similar values to control. Modulus of elasticity: similar values to control.	3% DMAHDM (by wt%): • Dead/alive bacteria: increased adherent compromised bacteria. • CFU: reduced the CFU by 3 log. Values between 10^5 and 10^7 (control between 10^8 and 10^{10}), in a biofilm of *P. gingivalis*, *P. intermedia*, *P. nigrescens*, *A. actinomycetemcomitans*, *F. nucleatum*, and *E. faecalis*. • Biofilm biomass: values between 0.3 and $0.6OD_{600}$nm (control between 0.9 and 1.8 OD_{600}nm) • Polysaccharide production: values between 10 and 20 mg/L (control between 40 and 100 mg/L).
Xie et al. (2016) [48]	3%MPC and 30%NACP	• 3% DMAHDM (by wt%): Flexural Strength: similar values to control. Modulus of elasticity: values lower than control.	3% DMAHDM (by wt%): • Dead/alive bacteria: increased adherent compromised bacteria. • Metabolic activity: approximate value $0A_{540}/cm^2$ (control between 0.6 and $0.7A_{540}/cm^2$). • CFU: reduced the CFU by 3 log. Values between 10^4 and 10^7 (control between 10^7 and 10^{10}), a biofilm of *S. mutans*, total Streptococci, and total microorganisms.
Zhang et al. (2017) [50]	3%MPC	• 1.5% DMAHDM (by wt%) after 180 days of immersion in water: Flexural strength: similar values to control. Modulus of elasticity: similar values to control.	1.5% DMAHDM (by wt%): • Dead/alive bacteria: increased adherent compromised bacteria. • Metabolic activity: on day 1 and after 180 days the values remained between 0.1 and $0A_{540}/cm^2$ (control between 0.8 and $1A_{540}/cm^2$). • Lactic acid production: on day 1 and after 180 days, the values remained between 0 and 2 mmol/L (control between 17 and 20 mmol/L). • CFU: reduced the CFU by 3 log. Values between 10 5and 10^7 (control between 10^7 and 10^{10}), a biofilm of *S. mutans,* total Streptococci, and total microorganisms.
Al-Dulaijan et al. (2018) [24]	20% NACP	• 3% DMAHDM (by wt%): Flexural strength: similar values to control. Modulus of elasticity: similar Values to control.	3% DMAHDM (by wt%): • Dead/alive bacteria: increased adherent compromised bacteria. • Metabolic activity: approximate value 0.05 A_{540}/cm^2 (control between 0.2 and 0.25 A_{540}/cm^2). • Lactic acid production: values between 0 and 1 mmol/L (control 5 mmol/L). • CFU: reduced the CFU between 3 and 4 log. Values between 10^3 and 10^7 (control between 10^5 and 10^9), in a biofilm of *S. mutans*, total Streptococci and total microorganisms.

Table 1 (*Continued*)

Authors	Material used together	Mechanical properties analyzed	Antibacterial properties
Cao et al. (2018) [35]	3% MPC	• 1.5% DMAHDM (by wt%): Flexural strength: similar values to control. Modulus of elasticity: similar values to control. 2.25% DMAHDM (by wt%): Flexural strength: similar values to control. Modulus of elasticity: similar values to control. The use of MPC did not change the properties when incorporated up to a maximum of 3%;	1.5 DMAHDM (by wt%): • Dead/alive bacteria: increased adherent compromised bacteria. • Metabolic activity: values between 0.05 and 0.1A_{540}/cm^2 (contro l0.25$A_{540/}cm^2$). Combined use with MPC resulted in approximately 0.05A_{540}/cm^2.
Wang et al. (2018) [51]	None	• Not analyzed	3% DMAHDM (by wt%): • Dead/alive bacteria: increased adherent compromised bacteria as the percentage of DMAHDM increased from 0.75–3%. • Metabolic activity: (48 h) Approximate value 0.4 OD_{540}/cm^2 (control 1.5OD_{540}/cm^2). (72 h). Approximate value 0.6 OD_{540}/cm^2 (control 1.6OD_{540}/cm^2). • Lactic acid production: (48 h) Approximate value 5 mmol/L (control 15 mmol/L). (72 h) approximate value 8 mmol/L (control 17 mmol/L). • Polysaccharide production: (48 h) Approximate value is 60 mg/mL (control 160 mg/mL). (72 h) approximate value is 100 mg/mL (control 230 mg/mL). • CFU: (48 h) Reduced CFU between 3 and 4 log. Values between 10^5 and 10^6. (72 h) Reduced CFU by 2 log. Approximate value 10^7 (control 10^9).
Wang et al. (2018) [60]	None	• Not analyzed	3% DMAHDM (by wt%): • *S. mutans, S. sanguinis,* and *S. gordonii* in planktonic forms did not develop resistance to DMAHDM dissolved in distilled water. • *S. mutans, S. sanguinis,* and *S. gordonii* in biofilm did not develop resistance to 3% DMAHDM incorporated into the resin. • CFU: reduced the CFU by 3 log. Approximate value 10^7 (control 10^{10}) in *S. mutans* biofilm. Reduced the CFU by 4 log. Values between 10^4 and 10^5 (control between 10^8 and 10^9) in *S. sanguinis* biofilm. Reduced the CFU by 4 log. The approximate value is 10^6 (control 10^{10}) in *S. gordonii* biofilm.
Chen et al. (2019) [44]	30% NACP	• 3% DMAHDM (by wt%): Flexural strength: similar values to control Modulus of elasticity: similar values to control • After 30 days of acid attack of the biofilm, composite with 3DMAHDM + 30NACP reached hardness values close to 2 GPa (control 1 GPa).	3% DMAHDM (by wt%): • Dead/alive bacteria: increased adherent compromised bacteria. • Metabolic activity: approximate value 0.1 OD_{540}/cm^2 (control 0.5 OD_{540}/cm^2). • Polysaccharide production: approximate value 0.1 OD_{490}/cm^2 (control 0.4 OD_{490}/cm^2). • Lactic acid production: approximate value 3 mmol/L (control18mmol/L). • CFU: reduced CFU by 4 log. Values between 10^4 and 10^5 (control between10^8 and10^9), *S. mutans* biofilm.
Wang et al. (2019) [37]	3%MPC + 20%NACP	• 3%DMAHDM (by wt%): Surface roughness: similar to control. The surface load density with the addition of DMAHDM was approximately 3 times higher than the controls.	3% DMAHDM (by wt%): • Dead/alive bacteria increased adherent compromised bacteria. DMAHDM isolated in a biofilm of 9 species resulted in more live bacteria compared to isolated species. • CFU: antibacterial efficacy of DMAHDM decreased as the number of species increased. Reduced CFU by 2 log. Values between 10^7 and 10^9 (control between 10^9 and 10^{10}). Biofilm of *P. gingivalis, S. gordonii, F. nucleatum, A. naeslundii, P. intermedia, A. actinomycetemcomitans, P. nigrensens, T. forsythia,* and *P. micra.* • Metabolic activity: value between0.4 and 0.8 OD_{492nm}/cm^2, (control between 1 and 1.4OD_{492nm}/cm^2). • Polysaccharide production: value between 20 and 60 mg/mL, (control between 60 and 80 mg/mL).
Xiao et al. (2019) [23]	0.12% silver nanoparticles (nAg); 3%MPC and 30%NFCA.	• 3% DMAHDM (by wt%): Flexural strength: similar values to control. Modulus of elasticity: similar values to control. Dentin shear bond strength: similar values to control.	3% DMAHDM (by wt%): • Dead/live bacteria: increased adherent compromised bacteria. • Metabolic activity: values between 0 and 0.2 OD_{540}/cm^2 (control between 0.8 and 1 OD_{540}/cm^2). • Polysaccharide production: values between 10 and 20 mg/L (control between 60 and 80 mg/L). • CFU: reduce the CFU between 3 and 5 log. Values between 10^5 and 10^6 (control between 10^8 and10^{10}), Biofilm of *P. gingivalis, A. actinomycetemcomitans* and *F. nucleatum.*

Table 1 (*Continued*)

Authors	Material used together	Mechanical properties analyzed	Antibacterial properties
Balhaddad et al., 2020 [43]	20% NACP	• 3% DMAHDM (by wt%): Flexural strength: superior values to commercial control and similar values to experimental control. Modulus of elasticity: values lower than controls Surface roughness: Similar values to controls. • 5% DMAHDM (by wt%): Flexural strength: similar values to commercial control. Modulus of elasticity: values lower than commercial control.	5% DMAHDM and 3%DMAHDM (by wt%): • Live/Dead: increased adherent compromised bacteria. • Metabolic activity: (3%DMAHDM) values between 0.05 and 0.1 A_{540}/cm^2. (5%DMAHDM) approximate value 0.05 A_{540}/cm^2 (control between 0.2 and 0.25 A_{540}/cm^2). • CFU: (3% DMAHDM) Reduced the CFU by 2 log. Values between 10^5 and 10^9. 5% DMAHDM reduced the CFU by 3 log. Values between 10^3 and 10^8 (control between 10^7 and 10^{10}) Biofilm of *S. mutans*, total Streptococci, total microorganisms, and total Lactobacilli. • Lactic acid: (3%DMAHDM) Approximate value 5 mmol/L (5%DMAHDM) Approximate value 1 mmol/L (Control 10 mmol/L).
Bhadila et al. (2020) [40]	20% NACP;	• 3% DMAHDM (by wt%): Flexural strengths: similar values to control. Modulus of elasticity: similar values to control. Surface roughness: similar values to control.	3% DMAHDM (by wt%): • Live/dead: increased adherent compromised bacteria. • CFU: reduced the CFU between 3 and 4 log. Values between 10^4 and 10^5 (Control 10^7) in S. *mutans* biofilm. • Lactic acid: approximate value is 2 mmol/L (control 10 mmol/L).
Bhadila et al. (2020) [64]	20% NACP; Low shrinkage-stress composite.	• 3% DMAHDM (by wt%): Flexural strengths: similar values to control. Modulus of elasticity: similar values to control. Hardness: similar values to control. Cytotoxicity: similar to the BisGMA monomer. Enamel hardness after an acid attack: the UV + 3DMAHDM + 20NACP + 43glass group had the highest values, followed by 3% DMAHDM alone.	3% DMAHDM (by wt%): • Not rated.
Bhadila et al. (2020) [41]	20% NACP Low shrinkage-stress composite.	• 3% DMAHDM (by wt%): Flexural strength: similar values to control. Modulus of elasticity: similar values to control. Dentin hardness: the group with DMAHDM + NACP + UV show 1.5 times greater than the control. • After 200 days of aging: Flexural strength: values lower than control. Modulus of elasticity: values lower than control.	3% DMAHDM (by wt%): • Live/Dead: increased adherent compromised bacteria. • CFU: reduced the CFU by 5 log. Approximate value 10^4 (Control 10^9) in *S. mutans* biofilm. • Lactic acid production: approximate value 2 mmol/L (control between 30 and 40 mmol/L). • Biomass: approximate value 1 OD = 570 (control 3.5 OD = 570).
Campos et al. (2020) [52]	dCHX	• Cytotoxicity at 1, 3, and 7 days: (5% DHAMDM) showed cytotoxicity values higher than chlorhexidine, exceeding the value of combined use on day 3. Flexural strength: values lower than control. Surface roughness: values lower than control.	5% DMAHDM (by wt%): • Metabolic activity: values between 0.11 A_{620}/cm^2 and 0.05 A_{620}/cm^2 (control between 0.36 A_{620}/cm^2 and 0.23 A_{620}/cm^2); biofilm of *S. mutans* and *C. albicans*, respectively.
Chen et al. (2020) [36]	None	Not rated	1.5% e 3% DMAHDM (by wt%): • Live/dead: increased adherent compromised bacteria. • Biomass: (1.5%DMAHDM) Values between 0.6 and 0.9 OD_{570nm}. (3%DMAHDM) Values between 0.3 and 0.6 OD_{570nm} (control between 1.5 and 1.8 OD_{570nm}). • Polysaccharide production: (1.5% DMAHDM) Approximate value 0.2 OD490 nm. (3%DMAHDM) values between 0.1 and 0.2 OD_{490nm} (control between 0.5 and 0.6 OD_{490nm}). • CFU: (1.5% DMAHDM) Reduced the CFU between 3 and 4 log. (3% DMAHDM) reduced the CFU between 5 and 6 log (control 10^9) in *S. mutans* biofilm. • Metabolic activity: (1.5% DMAHDM) 0.4 OD540 nm. (3%DMAHDM) 0.2 OD_{540nm} (control values between 1 e 1.2 OD_{540nm}). • Lactic acid: (1.5%DMAHDM) values between 4 and 8 mmol/L. (3%DMAHDM) approximately 2 mmol/L (Control 20 mmol/L).

Table 1 (*Continued*)

Authors	Material used together	Mechanical properties analyzed	Antibacterial properties
Mitwalli et al., 2020 [53]	3% MPC e 15% nanoparticles of calcium fluoride (nCaF2)	• 3%DMAHDM (by wt%): Flexural strength: similar values to control. Modulus of elasticity: similar values to control.	3% DMAHDM (by wt%): • Live/dead: increased adherent compromised bacteria. • CFU: reduced the CFU by 6 log. Values between 10^2 and 10^4. (Control between 10^4 and 10^{10}) biofilm of *S. mutans*, total Streptococci and total microrganisms. • Metabolic activity: approximate value 0 A_{540}/cm^2 (control between 0.15 and 0.2 A_{540}/cm^2). • Lactic acid: approximate value 0 mmol/L (control between 1 and 10 mmol/L).
Zhou et al. (2020) [38]	30% NACP	• 3%DMAHDM (by wt%): Flexural strength: similar values to control. Modulus of elasticity: similar values to control.	3% DMAHDM (by wt%): • Live/dead: increased adherent compromised bacteria. • Metabolic activity: approximate value 0.1 OD_{490}/cm2 (Control between 0.1 and 0.5 OD_{490}/cm^2). • CFU: reduced the CFU by 5 log. Approximate value 10^4 (control 10^9) for biofilm of *S.mutans* and multispecies. • Polysaccharide production: approximate value 0.1 OD_{450}/cm^2 (Control between 0.1 and 0.6 OD_{450}/cm^2. • Latic acid: approximate value 0 mmol/L (Control between 1 e 25 mmol/L).
Zhou et al. (2020) [39]	30% NACP	• 3%DMAHDM (by wt%): Flexural strengths: similar values to control Modulus of elasticity: similar values to control	3% DMAHDM (by wt%): • Live/dead: increased adherent compromised bacteria. • Metabolic activity: approximate value 0.10 OD_{450}/cm^2 (Control between 0.15 and 0.17 OD_{450}/cm^2). • CFU: reduced the CFU by 2 log. Approximate value 10^6 (Control between 10^8 and 10^9) in saliva-derived biofilm. • Lactic acid: values between 2 and 3 mmol/L (control 7 mmol/L). • Polysaccharide production: approximate value 0.2 OD_{490}/cm^2 (control 0.5 OD_{490}/cm^2).
Zhou et al. (2020) [42]	30% NACP	• 3%DMAHDM (by wt%): Flexural strengths: similar values to control. Modulus of elasticity: similar values to control. Enamel hardness: highest values for NACP + DMAHDM, followed by the group with only DMAHDM (1.5 times greater than the control).	3% DMAHDM (by wt%): • Live/Dead 48 h: increased adherent compromised bacteria. • Metabolic activity: DMAHDM greatly reduced the metabolic activity of biofilms. Approximate value 0.1 OD_{450}/cm^2 (control close to 0.5 OD_{450}/cm^2). • CFU: reduced the CFU by 5 log. Values between 10^3 and 10^4 (Control 10^8). *S. mutans* biofilm. • Lactic acid: approximate value 0 mmol/L (control 12 mmol/L). • Polysaccharides: values between 30 and 40 mg/mL (Control 230 mg/mL).

biofilm against a mass fraction of 3%DMAHDM dissolved in sterile distilled water or incorporated in resin composites also containing bisphenol A glycidyl dimethacrylate (BisGMA) and triethylene glycol dimethacrylate (TEGDMA). The results showed that none of the species studied developed resistance in the planktonic form of biofilm, even after 20 passages of tests that took about four months.

One of the main concerns when using antibacterial compounds in resin-based dental materials is bacterial resistance induction since the material should remain in the oral cavity long-lastly. Wang et al. (2017) [61] dissolved DMAHDM in sterile distilled water at 200 g/mL and examined the induction of drug resistance in eight species of cariogenic, endodontic, and periodontal bacteria (*S. mutans, S. sanguis, S. gordonii, E. faecalis, A. actinomycetemcomitans, F. nucleatum, P. gingivalis, and P. intermedia*). The results showed that after ten passages, the bacterial strains did not develop resistance to the monomer in contrast with chlorhexidine that induced resistance in four species, indicating a low risk for DMAHDM induce resistance.

However, as highlighted by the authors in the study of Wang et al. (2018) [60], only Streptococcal species were examined, against a mass fraction of 3% DMAHDM, incorporated in a resin composite. Therefore, allied to the fact that only that study was identified in our search, it remains unclear if the monomer stimulates resistance in different conditions against other species used in a resin composite for dental restoration.

4. Cytotoxicity

Campos et al. (2019) [52] synthesized a self-curing experimental acrylic resin and analyzed the toxicity against rat fibroblasts by adding eluted resin samples in the medium of Dulbecco Modified Eagle Medium with fibroblast culture. The DMAHDM was incorporated in the PMMA acrylic resin (5 wt%), along with polymethyl methacrylate polymer, benzoyl peroxide (initiator), pigments, methyl methacrylate monomer, cross-linking agent (EDMA), DMT, inhibitor, and fluorescent. The authors observed morphological changes in the cells and higher cytotoxicity than chlorhexidine diacetate (dCHX). Similarly, Regis et al. (2012) [62] showed higher cytotoxicity values for MUPB (methacryloyloxyloxyurelpyridinium bromide), a QAM also used in acrylic resin, compared to the control. The authors highlighted that the cytotoxicity values found for MUPB are close to the results for HEMA and UDMA, monomers widely used in restorative dental materials [63].

5. Mechanical properties

Resin-based dental materials are used in the oral cavity for restorative applications, subjected to occlusal forces, temperature cycles, and erosive challenges from the diet and biofilm. Thus, tooth restorations' long-lasting clinical behavior depends on the obtention of material with satisfactory mechanical strength. Most of the studies analyzed [23–25,35,37–50,52,53,64] have verified properties of experimental composites, such as flexural strength (FS), modulus of elasticity (ME), fracture toughness (FT), surface roughness (SR), hardness (HC), dentin shear bond strength (DSBS) and color stability (CS).

The majority of studies that analyzed resin composites observed no change in FS with an addition of 1.5 to 3.75 wt% DMAHDM [23–25,38–44,46–50,53]. However, Balhaddad et al. (2020) [43] observed a reduction of FS by the addition of 5 wt% DMAHDM com-

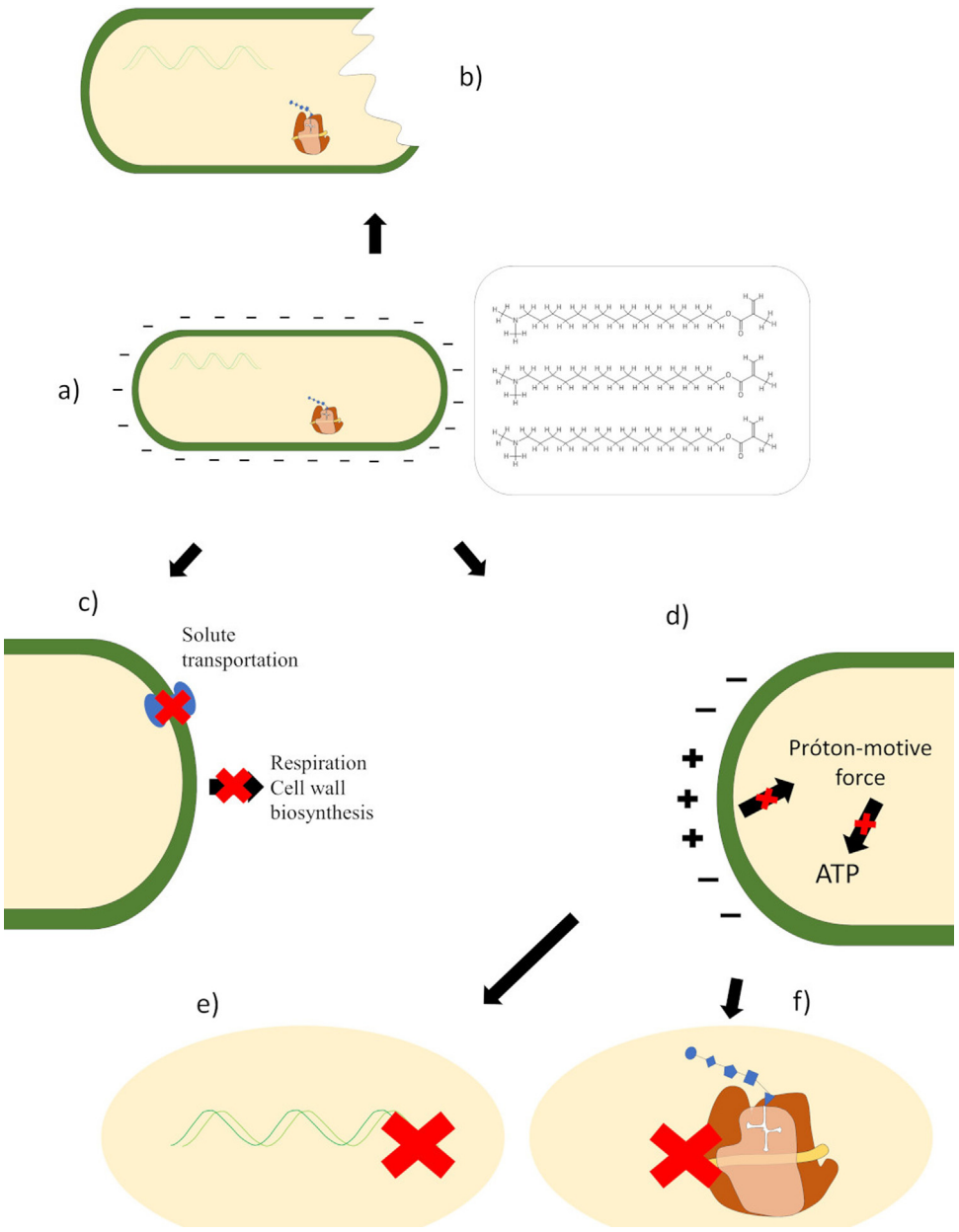

Fig. 2. Mechanism of action of DMAHDM. (a) The contact between the negatively charged bacteria and long-chained positively charged quaternary ammonium compounds can lead to (b) Penetration of bacterial membrane, causing cytoplasmatic leakage; (c) Disruption of membrane functions, such as solute transportation, respiration, and cell wall biosynthesis; and (d) Alteration on the superficial electrostatic charge from the contact with the positive charge on quaternary amine N+ of the monomer, collapsing proton-motive force and ATP production, leading to (e) Loss of DNA multiplication capability and (f) Interruption of protein synthesis.

pared to a resin composite with 3 wt% DMAHDM and experimental control, although it remained superior to the commercial control.

For ME, the experimental resin composites showed a reduction in the studies of Xie et al. (2016) [48], Mitwalli et al. (2020) [53], and Balhaddad et al. (2020) [43]. Xie et al. (2016) [48] worked with DMAHDM at a mass fraction of 1.5% to 3%, observing a significant reduction compared to the control. The work of Mitwalli et al. (2020) [53] showed a reduction with 3% (by wt%) DMAHDM in comparison with the experimental control, but it remained similar to the commercial control. Balhaddad et al. (2020) [43] obtained results indicating ME significantly lower than the control by adding 3% and 5%DMAHDM mass fractions. These results contrast with other studies in which there was no change [23–25,38–42,44,46,47,49,50,64].

Surface roughness was analyzed in three studies [37,40,43] by the addition of 3 wt% DMAHDM [37,40,43] and 5 wt% [43]. All results demonstrated no alterations in SR, even after biofilm challenges [43], showing that adding up to 5 wt% did not alter that property. DSBS, FT, and HC of experimental resin composites were also verified by Xiao et al. [23], Wu et al. [45], and Bhadila et al. (2020) [64], respectively. The results showed that 3 wt% DMAHDM in the experimental resin composites did not change the properties tested.

In comparison, the studies that examined acrylic resins [35,52] observed decreased FS, ME, SR, and color stability by the addition of 2.25 wt% DMAHDM [35] and 5 wt% DMAHDM [52]. It is interesting to highlight the fact that the addition of 1.5% (by wt%) DMAHDM resulted in similar FS and ME compared to control in the work of Cao et al. [35].

The resin composites' physical and mechanical properties can critically affect performance in the oral cavity [65]. The properties investigated in this review's studies may influence the ability to

resist occlusal forces, the rigidity of the material, and the ability to accumulate biofilm, thus resulting in better or worse longevity [65–67]. The results suggest that these properties were maintained for composite resins in most studies and are comparable to commercial control materials. Therefore, the use of DMAHDM does not appear to weaken the restorative material, keeping its benefit to impair bacterial growth.

6. Association with other chemical components

The use of DMAHDM monomer generally occurred in conjunction with other anti-adherent, antibacterial, and remineralize components: 2-methacryloyloxyethyl phosphorylcholine (MPC), nanoparticles of amorphous calcium phosphate (NACP), and nanoparticles of calcium fluoride (nCaF2), chlorhexidine diacetate (dCHX), silver nanoparticles (Ag), and nanoparticles of calcium fluoride [23,52,53].

6.1. MPC

Special attention should be given to MPC in such an analysis, considering that several studies have shown better antibacterial performance using DMAHDM + MPC (Table 1). Wang et al. (2019) [37], using multispecies periodontal biofilm, demonstrated that the efficacy of DMAHDM alone was reduced as the biofilm changed from single to multiple species. However, the compound with DMAHDM + MPC maintained its efficacy, even with an increase in pathogens, suggesting that the combined use is promising.

These can be explained initially by MPC's hydrophilic nature since most proteins adsorb preferably to hydrophobic surfaces [68–70]. Moreover, it is known that the abundance of free but not water-bound water around MPC polymers is capable of detaching proteins on the surface, preventing adsorption [71–73]. Thus, by repelling the layer of salivary proteins that naturally deposits on the resin-based material's surface, MPC contributes to increasing the interaction between DMAHDM and bacteria, which reinforces the antibacterial effect [19,74–77].

6.2. NACP

Considering NACP, it is remarkable that Ca and P ions' release provides a mechanism to inhibit caries lesions, especially during an acidic cariogenic challenge, helping to maintain the pH to avoid demineralization and facilitating remineralization [48,78,79]. The combination of NACP and DMAHDM was examined in several studies [23–25,37–45,48,49,64], and the combined use did not impair Ca and P ions release. Thus, the association between DMAHDM and NACP can be beneficial due to the synergistic effect of antibacterial activity, remineralization, and acid neutralization. Some studies demonstrated changes in enamel hardness [42,64] and dentin hardness [41] in resin composites with 3 wt% DMAHDM and 20 wt% or 30 wt% NACP, showing hardness 1.5 [41] and two times [42,64] greater in comparison to the control. However, an observation should be made, because according to the data collected, the concentration of NACP should not exceed 30% due to the risk of damaging mechanical properties, as was demonstrated by Chen et al. (2019) [44].

6.3. nAg

The nAg nanoparticles have antibacterial activity due to their ability to increase cytoplasmic permeability, which facilitates bacterial' cell envelope breaking [71]. Therefore, dental materials containing Ag nanoparticles can interfere with biofilm formation to prevent dental caries [80]. Xiao et al., 2019 [23] analyzed the antibacterial activity of 3%DMAHDM + 0.12%nAg + 3%MPC and obtained increased antibacterial effect through the reduction in polysaccharide production, metabolism, adhered live bacteria, and CFU count, without harming mechanical properties such as modulus of elasticity, flexural strength, and shear bond strength in dentin. However, as it is known that MPC improves the ability of DMAHDM to inhibit bacterial activity [19,74–77], we cannot rule out the possibility that MPC contributed to increasing the antibacterial effect of the blend.

6.4. dCHX

The combined use of DMAHDM with dCHX [52] in PMMA acrylic resin showed no benefit compared to DMAHDM alone. dCHX has been widely used in adhesive systems to improve the restoration's longevity by inhibiting matrix metalloproteinases (MMPs) and having antimicrobial efficacy [81–83]. However, this component has some flaws, such as increased water sorption [84]. Campos et al. [52] observed that the combined use resulted in worse surface roughness values, so it does not seem as if this approach was more beneficial than DMAHDM alone.

6.5. Nanoparticles of calcium fluoride ($nCaF_2$)

Topical fluoride can stabilize the pH of saliva, reduce cariogenic bacteria, remineralize dental structure, and strengthen enamel, minimalizing demineralization because of fluorapatite crystals formation [85–87]. Calcium fluoride nanoparticles would enforce the enamel structure by promoting remineralization and forming fluorapatite [53].

Our research identified a single study regarding the combined use of nCaF2 and DMAHDM. Mitwalli et al. (2020) [53] observed flexural strength and modulus of elasticity similar to control by adding 15% nCaF2 + 3%DMAHDM mass fraction. However, the 15%nCaF2+ 3%DMAHDM + 3%MPC group showed a significant reduction in FS compared to the control. The association achieved great antibacterial activity evidenced by the reduction in CFU count, metabolic activity, and lactic acid production, allied with fluoride and calcium ion release, further potentialized by association with 3%MPC mass fraction. The results indicate the potential to use such a combination in dental restorative to prevent recurrent carious lesions. However, more studies must analyze the alliance to understand the effects of long-term ions release and mechanical properties.

7. Limitations and future perspectives

It was not possible to identify clinical studies using any material containing DMAHDM, so the notes made here are limited to *in vitro* studies. Moreover, only the papers by Zhang et al. (2017) [50] and Bhadila et al. (2020) [41] evaluated the long-term efficacy of 1.5 wt% to 3 wt% DMAHDM, obtaining consistent results for antibacterial activity after 90 [41] and 180 days [50]. However, regarding mechanical properties, Zhang et al. (2017) [50] obtained results similar to the control for FS and ME after 180 days, contrasting with Bhadila et al. (2020) [41] that observed a similar reduction for FS, but worse values for ME compared to the control after 200 days. Thus, the results presented in this review were analyzed only for the monomer's immediate efficacy because other long-term studies could not be identified.

Future studies are needed to obtain further clarification on the development of oral cell cytotoxicity and bacterial resistance. Materials with different concentrations of DMAHDM exposed to more bacterial species should be analyzed, as the oral environment is composed of a very diverse microbiota, consisting of more than 700 bacterial species [88]. It is pertinent to recommend evaluating the bactericidal capacity of DMAHDM and the possibility to

favor commensal species over cariogenic species. For better safety in maintaining mechanical properties, analysis of other resin composites' characteristics such as CS, FT, and SR should be carried out, considering that most studies have observed only FS and ME.

8. Conclusion

The DMAHDM monomer has demonstrated short-time antibacterial efficacy against several oral pathogenic species and its benefits by association with MPC. The experimental resin composites containing DMAHDM showed satisfactory FS and ME, but this monomer worsened acrylic resins' mechanical properties. DMAHDM presents itself as a promising antibacterial monomer for association with components such as MPC, nCaF^2 and NACP in resin-based dental materials. Future research is still needed to provide more information regarding cytotoxicity, the development of bacterial resistance, the capability of favoring commensal species over cariogenic, and the analysis of mechanical properties, such as fracture toughness, surface roughness, and color stability.

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Conflicts of interest

None declared.

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