DMAHDM Nanocomposite

Subjects: Biochemical Research Methods
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Researchers have developed novel nanocomposites that incorporate additional biomaterials with dimethylaminohexadecyl methacrylate (DMAHDM) in order to reduce secondary caries. The aim of this review was to summarize the current literature and assess the synergistic antibacterial and remineralizing effects that may contribute to the prevention of secondary caries. An electronic search was undertaken in MEDLINE using PubMed, Embase, Scopus, Web of Science and Cochrane databases.

biomaterial

1. Introduction

Dental composite resins are widely used restorative materials due to their ability to conserve tooth structure during cavity preparation, aesthetics and direct-filling capabilities [1][2][3][4]. However, composite resin restorations still present several drawbacks including polymerization shrinkage, bulk fracture, mechanical fatigue by mastication, marginal leakage and biodegradation by acid while also being challenged by the adhesion and accumulation of cariogenic bacteria (Viz., *Streptococcus mutans* and *Lactobacilli*) when compared to other materials used for restoration [1][2][5].

Dental biofilm adjoining to the tooth-restoration margin could lead to secondary caries, the primary reason for failure of composite restorations [4], accounting for nearly 50% of failures within 10 years [6][7][8]. The replacement of failed restorations may result in further tooth structure removal, which can weaken the remaining hard tissues and affect the long-term prognosis of the tooth [9]. Consequently, efforts have been made to create a novel dental composite resins with the addition of antibacterial and remineralizing agents to combat secondary caries [1].

When copolymerized in resins, quaternary ammonium methacrylates (QAMs), which are cationic compounds, exhibit low toxicity and a broad-spectrum antimicrobial effect [2][10][11][12]. These positively charged methacrylic monomers bind and disrupt the electrical equilibrium of the negatively charged bacterial membranes thereby causing rupture and cell death [13].

The antibacterial efficacy of QAMs has been correlated with the alkyl chain length (CL) of the hydrocarbons as this causes an increase in hydrophobicity [2][14][15]. Previous studies have reported that an increase in CL from 3 to 16 greatly enhanced the efficacy of dental materials against bacteria [16], which then decreased at a CL of 18 [17][18]. A CL of 18 exhibited an increase in live bacteria and biofilm thickness and did not further decrease the metabolic activity or strengthen the bacterial inhibition effect compared to a CL of 16 [19][20]. Therefore,

dimethylaminohexadecyl methacrylate (DMAHDM) monomer with a CL of 16 was found to exhibit the best antiseptic effect against oral bacterial pathogens when used as bonding agents, sealers or other dental materials [17][21].

Due to the strong antibacterial properties of DMAHDM, researchers have experimented with combining additional biomaterials with DMAHDM to explore any synergistic mechanisms of action to increase the efficacy of DMAHDM and reduce biofilm formation at the tooth-restoration interface [8][11]. DMAHDM does not possess any inherent remineralizing capabilities, so efforts have also been made to incorporate biomaterials that promote tooth remineralization as another approach for caries inhibition [1][8][22]. Furthermore, the development of this nanotechnology is quickly evolving and there has been a shift in focus to create novel composite resins that possess both antibacterial and remineralizing capabilities [23].

Despite this growing area of research, there is yet to be a review that outlines the current combinations of DMAHDM nanocomposite or any synergistic mechanisms of action that enhance the effects of DMAHDM nanocomposite. As such, this systematic review intended to summarize the literature on dental composite resins that incorporate additional biomaterials with DMAHDM, and to assess for any possible synergistic antibacterial and remineralizing effects that may aid in the prevention of secondary caries.

2. Study Selection

The initial literature search retrieved 954 results, 559 remained after removal of duplicates. After screening of titles and abstracts 25 articles remained, of which 15 were eligible for inclusion after the full-text review. Six articles were excluded as they did not use DMAHDM or had an additive biomaterial [24][25][26][27][28][29], one each used dental materials other than the composite [30] or tested materials with compromised mechanical properties [31] while two were non-English articles [32][33]. The results of the screening and search process are presented in Figure 1.

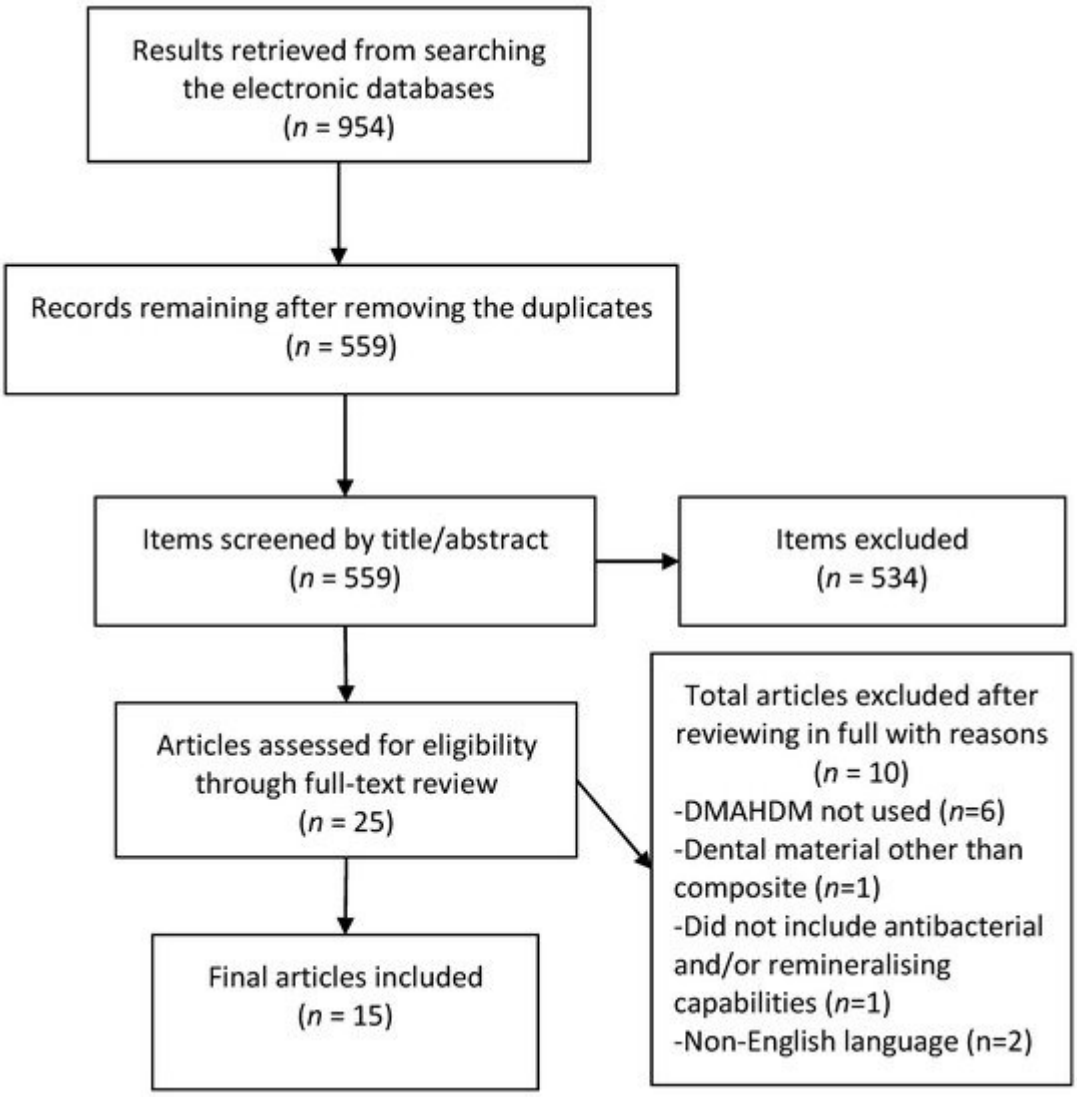

Figure 1. Preferred reporting items for systematic review and meta-analyses (PRISMA) flow chart demonstrating the selection of articles.

3. Study Characteristics

All study characteristics are reported in Supplementary Materials Table S1. There were no studies published prior to 2015. All the studies were conducted in USA and/or China, or Japan. Eight of the studies used EBPM (ethoxylated bisphenol A-dimethacrylate (EBPADMA) and pyromellitic glyceroldimethacrylate (PMGDM)) as the composite resin matrix while seven of the studies used a BisGMA-TEGDMA (bisphenol A-glycidyl methacrylate and triethylene glycol dimethacrylate) resin matrix. Nine studies used the heliomolar commercial composite as a control comparison group, two studies used the Renamel Microfill commercial composite and one study used both as a control group.

There were six different combinations reported in the literature, which incorporated additional biomaterials with DMAHDM: DMAHDM + nanoparticles of amorphous calcium phosphate (NACP); DMAHDM + 2-methacryloyloxyethyl phosphorylcholine (MPC); DMAHDM + NACP + MPC; DMAHDM + NACP + silver

nanoparticles (AgNPs); DMAHDM + NACP + MPC + AgNPs and DMAHDM + NACP + MPC + AgNPs + polyamidoamine dendrimers (PAMAM).

All studies used a modified Menschutkin reaction method to synthesize DMAHDM [2]. NACP was synthesized with a spray-drying technique [34]. MPC was sourced commercially. AgNPs was chemically prepared by dissolving silver 2-ethylhexanoate in 2-(tert-butylamino)ethyl methacrylate (TBAEMA) [35]. PAMAM was used at 1 mg/mL concentration, by mixing PAMAM in distilled water [23]. Antibacterial and remineralizing outcomes used by the included studies are outlined in Table 1.

Table 1. Tests for antibacterial and remineralizing outcomes.

Antibacterial Outcomes	Remineralizing Outcomes
• Colony-forming units (CFU): mean differences in CFU count of biofilms on experimental and control composites • Lactic acid production: mean differences in lactate concentrations (mmol/L) of biofilms on experimental and control composites • Metabolic activity (MTT): mean differences in optical density 450/cm^2 of biofilms on experimental and control composites • Biofilm culture medium pH: mean differences in pH of culture mediums on experimental and control composites • Live/dead assay: images taken at random (qualitative variable) of bacteria on experimental and control composites • Polysaccharide production: mean difference in optical density 450/cm^2 of experimental and control composites	• Calcium and phosphate ion concentrations: mean difference in calcium and phosphate ion concentrations (mmol/L) of experimental and control composites immersed in a solution • Dentine hardness: mean differences in GPa (gigapascals) of root surfaces adjacent to experimental and control composites

4. Risk of Bias

As reported in Table 2, all 15 studies demonstrated a medium risk of bias and reported an adequate control group, standardized sample production process, standardized antibacterial/remineralizing assessment and adequate

statistical analysis. No studies reported that the antibacterial or remineralizing properties were evaluated by a single operator or presented a sample size calculation. No studies reported about the manufacturer's instructions for using the materials. As these are experimental studies, most studies prepared their own composite materials.

Table 2. Risk of bias among the included studies.

Reference	Sample Size Calculation	Adequate Control Group	Use of Materials According to Manufacturer's Instruction	Standardized Sample Production Process	Standardized Antibacterial/ Remineralizing Assessment	Evaluation of Antibacterial/ Remineralizing Properties by a Single Operator	Adequate Statistical Analysis	Risk of Bias *
Wu et al., 2015 [34]	No	Yes	No	Yes	Yes	No	Yes	Medium risk
Wu et al., 2015 [36]	No	Yes	No	Yes	Yes	No	Yes	Medium risk
Zhang et al., 2015 [37]	No	Yes	No	Yes	Yes	No	Yes	Medium risk
Melo et al., 2016 [38]	No	Yes	No	Yes	Yes	No	Yes	Medium risk
Wang et al., 2016 [39]	No	Yes	No	Yes	Yes	No	Yes	Medium risk
Wang et al., 2016 [40]	No	Yes	No	Yes	Yes	No	Yes	Medium risk
Xie et al., 2016 [41]	No	Yes	No	Yes	Yes	No	Yes	Medium risk
Zhang et al., 2016 [20]	No	Yes	No	Yes	Yes	No	Yes	Medium risk
Xiao et al., 2017 [23]	No	Yes	No	Yes	Yes	No	Yes	Medium risk
Zhang et al., 2017	No	Yes	No	Yes	Yes	No	Yes	Medium risk

Reference	Sample Size Calculation	Adequate Control Group	Use of Materials According to Manufacturer's Instruction	Standardized Sample Production Process	Standardized Antibacterial/ Remineralizing Assessment	Evaluation of Antibacterial/ Remineralizing Properties by a Single Operator	Adequate Statistical Analysis	Risk of Bias *
[42]								
Al-Dulaijan et al., 2018 [1]	No	Yes	No	Yes	Yes	No	Yes	Medium risk
Wang et al., 2019 [43]	No	Yes	No	Yes	Yes	No	Yes	Medium risk
Xiao et al., 2019 [35]	No	Yes	No	Yes	Yes	No	Yes	Medium risk
Bhadila et al., 2020 [2]	No	Yes	No	Yes	Yes	No	Yes	Medium risk
Zhou et al., 2020 [44]	No	Yes	No	[45] Yes	Yes	No	Yes	Medium risk

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restorations. One study did not specify the microorganisms tested.

Table 3. Antibacterial results.

Reference	Biomaterial Combinations Used and Comparison Group/s	Microorganisms Tested	Time Points Assessed	Methodology Used to Assess Antibacterial Effectiveness	Results Summary
Wu et al., 2015 [37]	(1–5) Five variants with 10% DMAHDM + 20% NACP + 0%, 2.5%, 5%, 7.5% or 10%, respectively, of microcapsules of formaldehyde and urea Comparison group: (6) 20% NACP	Total microorganism Total streptococci *Streptococcus mutans*	2 days	Live/dead assay	Biofilm was primarily alive in (6) (1) showed mostly dead bacteria.
				Metabolic assay (MTT)	Metabolic activity of (1) was reduced by 96% compared to (6)
				Production of Lactic Acid	(1) reduced lactic acid production by 99% compared to (6)
				CFUc counts	(1) reduced the biofilm CFU by 3–4 times compared to (6)

Reference	Biomaterial Combinations Used and Comparison Group/s	Microorganisms Tested	Time Points Assessed	Methodology Used to Assess Antibacterial Effectiveness	Results Summary
Wu et al., 2015 [39]	(1–4) Four variants with 20% NACP and DMAHDM mass fraction of 0.75%, 1.5%, 2.25% and 3%, respectively Comparison group: (5) 20% NACP	Total microorganisms Total streptococci *S. mutans*	2 days	Live/dead assay	Biofilm was primarily alive in (5). Amounts of dead bacteria increased with increase in the mass fraction of DMAHDM
				Metabolic assay (MTT)	Metabolic activity—(4) was 96% lower than (5)
				Production of Lactic Acid	Increasing DMAHDM mass fraction caused a monotonic decrease in the production of lactic acid
				CFUc counts	Antibacterial activity increased and CFU decreased with increase in the mass fraction of DMAHDM
Zhang et al., 2015 [40]	(1) 1.5% DMAHDM (2) 3% MPC (3) 1.5% DMAHDM + 3% MPC Comparison groups: (4) 0% DMAHDM + 0% MPC (5) Heliomolar commercial composite	Total microorganisms Total streptococci *S. mutans*	2 days	Live/dead assay	(1) demonstrated lower bacterial adhesion but most bacteria were alive (1) showed high amounts of dead bacteria
				Metabolic assay (MTT)	(3) had the least metabolic activity and higher reduction in biofilm growth compared to (1) or (2)
				Production of Lactic Acid	(3) had the least lactic acid production
				CFU counts	(3) reduced CFU counts by >3 logs compared to (4) or (5) and had much less biofilm CFU than (1) or (2)
				Protein adsorption	(3) had less protein adsorption compared to

Reference	Biomaterial Combinations Used and Comparison Group/s	Microorganisms Tested	Time Points Assessed	Methodology Used to Assess Antibacterial Effectiveness	Results Summary
					controls than (1) or (2)
Melo et al., 2016 [41]	(1) 5% DMAHDM + 0.1% AgNPs + 30% NACP Comparison group: (2) 0% DMAHDM + 0% AgNPs + 0% NACP	Did not specify	7 days	Live/dead assay	Dental materials containing multiagents resulted in compromised bacteria at tooth-composite interface
Wang et al., 2016 [42]	(1) 3% DMAHDM + 20% NACP Comparison groups: (2) 20% NACP (3) Heliomolar commercial composite	*P. gingivalis, P. intermedia, Prevotella nigrescens, A. actinomycetemcomitans, F. nucleatum, Enterococcus faecalis*	2 days	Live/dead assay	(1) mainly had dead bacteria while (2) and (3) had primarily live bacteria
				CFU counts	(1) CFU reduction differed between the bacterial species differently, few by <3 log while others by >3 log
				Crystal violet biofilm biomass assay	(1) had a significantly decreased biomass value compared to (2) and (3)
Wang et al., 2016 [43]	(1) 3% DMAHDM (2) 3% MPC (3) 3% DMAHDM + 3% MPC [b] Comparison groups: (4) 0% DMAHDM + 0% MPC (5) Heliomolar	*P. gingivalis, P. intermedia, A. actinomycetemcomitans, F. nucleatum*	2 days	Polysaccharide production	(1) had greatly reduced polysaccharide production for all six species compared to (2) and (3)
				Live/dead assay	(2) reduced the adhesion of bacteria, (3) demonstrated mostly dead bacteria
				Metabolic activity (MTT)	(3) presented lower biofilm metabolic activity on all the tested periodontal pathogens compared to (4) and (5)

Reference	Biomaterial Combinations Used and Comparison Group/s	Microorganisms Tested	Time Points Assessed	Methodology Used to Assess Antibacterial Effectiveness	Results Summary
	commercial composite			CFU counts	Addition of DMAHDM or MPC independently into the composite decreased the CFU
				Protein adsorption	(1) had no effect on protein adsorption (2) substantially decreased the protein adsorption by one log compared to (4) and (5)
				Polysaccharide production	(3) had much less polysaccharide production compared to (4) and (5)
Xie et al., 2016 [44]	(1) 30% NACP + 3% MPC (2) 30% NACP + 3% MPC + 1.5% DMAHDM (3) 30% NACP + 3% MPC + 3% DMAHDM	Total microorganisms Total streptococci *S. mutans*	2 days (with 2-day biofilm) and 4 days (pH required 72 h of incubation)	Live/dead assay	(4) and (5) were completely covered by live bacteria. Bacterial adhesion was reduced by MPC, DMAHDM produced an antibacterial effect. (3) had the most dead bacteria followed by (2) and (1)
	Comparison groups: (4) 30% NACP (5) Heliomolar commercial composite			Metabolic assay (MTT)	Metabolic activity of biofilms of (3) < (2) < (1) (3) had the lowest metabolic activity of biofilms among all
				CFU counts	(3) had the least biofilm CFU, count reduced by 3 logs compared to (4) and (5).
				Protein adsorption	(1) had protein adsorption one log less than (5); (2) and (3) had no effect on the protein adsorption
				pH of biofilm culture	(3) maintained a pH above 6.5.

Reference	Biomaterial Combinations Used and Comparison Group/s	Microorganisms Tested	Time Points Assessed	Methodology Used to Assess Antibacterial Effectiveness	Results Summary
				medium	
Zhang et al., 2016 [20]	(1–5) Five variants with 20% NACP with QAM CL of 3, 6, 12, 16 and 18, respectively Comparison groups: (6) 20% NACP (7) Renamel Microfill commercial composite	Total microorganisms Total streptococci *S. mutans*	30 days	Live/dead assay	(6) and (7) were covered by live bacteria. Dead bacteria increased progressively from CL3 up to CL16 with maximum antibacterial potency at CL16 before decreasing in potency at CL18 as indicated by some live bacteria.
				Metabolic assay (MTT)	Metabolic activity of biofilms decreased with increase in CL from 3 to 16. CL16 had maximum reduction on metabolic activity, which remained constant with any increase in CL
				Production of Lactic Acid	The biofilms on (6) and (7) produced the most acid. Acid production capacity of biofilm increased with an increase in CL from 3 to 16 CL16 minimized lactic acid production by 10-fold compared to (6) and (7)
Zhang et al., 2017	(1) 1.5% DMAHDM	Total microorganisms Total streptococci	185 days	CFU counts	CFU counts decreased with an increase in the CL from 3 to 16. Antibacterial activity was strongest at CL16, which lowered at C18. CL16 reduced all three CFU counts by 2 logs compared to (6) and (7)
				Live/dead assay	(3) had high levels of dead bacteria and lower

Reference	Biomaterial Combinations Used and Comparison Group/s	Microorganisms Tested	Time Points Assessed	Methodology Used to Assess Antibacterial Effectiveness	Results Summary
[45]	(2) 3% MPC (3) 1.5% DMAHDM + 3% MPC Comparison group: (4) Heliomolar commercial composite	*S. mutans*			bacterial attachment. Protein-repellent and anti-biofilm activities remained same from day 1 to 180
				Metabolic assay (MTT)	(1) and (2) showed higher reduction of biofilm viability than (4) (3) had the least metabolic activity Antibacterial function remained the same from day 1 to 180, being unimpacted by water-aging
				Production of Lactic Acid	(3) had the least lactic acid production
				CFU counts	(1) and (2) decreased the CFU compared to (4). (3) had greater antibacterial properties compared to (1) and (2) and was nearly 3 logs lower than (4), both at 1 day and 180 days of water-aging ($p < 0.05$).
				Protein adsorption	MPC greatly inhibited protein adsorption with no difference between 1 day and 180 days. (3) had the same protein adsorption as (2) ($p > 0.1$), which was about one tenth that of (4) and (1) ($p < 0.05$).
Al-Dulaijan et al., 2018 [1]	(1) 20% NACP + 3% DMAHDM (2) 20% NACP Comparison group:	Total microorganisms Total streptococci *S. mutans*	2 days	Live/dead assay	(1) had much less live bacteria compared to (2) and (3).
				Metabolic assay (MTT)	(1) greatly decreased the metabolic activity of the biofilms compared to

Reference	Biomaterial Combinations Used and Comparison Group/s	Microorganisms Tested	Time Points Assessed	Methodology Used to Assess Antibacterial Effectiveness	Results Summary
	(3) Heliomolar commercial composite				(2) and (3) ($p < 0.05$). (2) had similar metabolic activity to (3) indicating that NACP had little effect on biofilm viability.
				Production of Lactic Acid	(1) had the least lactic acid production.
				CFU counts	(1) decreased all three CFU counts by 3–4 logs compared to (2) and (3).
Wang et al., 2019 [46]	(1) 3% MPC + 20% NACP (2) 3% DMAHDM + 20% NACP (3) 3% DMAHDM + 3% MPC + 20% NACP Comparison groups: (4) 20% NACP (5) Heliomolar commercial composite	(1) Biofilm with one species: *P. gingivalis* (2) Biofilm with three species: *P. gingivalis, S. gordonii* and *F. nucleatum* (3) Biofilm with six species: *P. gingivalis, S. gordonii, F. nucleatum, A. naeslundii, P. intermedia* and *A. actinomycetemcomitans* (4) Nine-species biofilm: *P. gingivalis, S. gordonii, F. nucleatum, A. naeslundii, P. intermedia, A. actinomycetemcomitans, P. nigrescens, Tannerella forsythia* and *Parvimonas micra*	4 days	Live/dead assay	(2) had large quantity of dead bacteria, (1) showed lower bacterial adhesion. (3) had large quantity of dead bacteria but lower bacterial adhesion that (4) and (5) which were largely covered by live bacteria
				Metabolic assay (MTT)	(1) and (2) reduced the metabolic activity greatly compared to (3) Killing power of DMAHDM decreased with increase in the number of species in the biofilm (3) had stronger killing efficacy on all biofilm types
				CFU	(3) had higher reduction of CFU than (1) and (2), by >3 log on all four biofilm types
				Protein adsorption	DMAHDM demonstrated no effect on protein adsorption (1) decreased the protein adsorption by approximately 1 log, compared to (2) and (5)

Reference	Biomaterial Combinations Used and Comparison Group/s	Microorganisms Tested	Time Points Assessed	Methodology Used to Assess Antibacterial Effectiveness	Results Summary
				Polysaccharide production	Single species biofilms produced less polysaccharides than multi species biofilms (1) and (2) decreased the amount of polysaccharide produced by biofilms, (3) showed least production in all the biofilm types
Xiao et al., 2019 [38]	(1) 30% NACP + 3% MPC + 3% DMAHDM (2) 30% NACP + 3% MPC + 3% DMAHDM + 0.12% AgNPs [a,c] Comparison groups: (3) 30% NACP (4) Renamel Microfill commercial composite [3][35][37][40][41][42]	(1) *P. gingivalis* (2) *A. actinomycetemcomitans* (3) *F. nucleatum*	2 days [40]	Live/dead assay	(1) and (2) had much less biofilms, with mostly dead bacteria compared to (3) and (4), which were mostly covered by live bacteria.
				Metabolic assay (MTT)	Metabolic activity of (1) and (2) lower than (3) and (4) (2) showed lower biofilm metabolic activity for all three bacteria species than (1)
				Production of Lactic Acid	(1) had significantly decreased CFU counts for all three species, (2) showed the lowest CFU. (1) reduced the CFU counts by 4 logs on *P. gingivalis* and *A. actinomycetemcomitans*, while (2) reduced the CFU counts by 5 log. (1) and (2) reduced the CFU counts for F. nucleatum, by 3 and 5 logs, respectively. [42]
				Protein adsorption	(1) and (2) decreased protein adsorption, it was e tenth of (3) and (4)

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l a decrease in CFU counts for all microorganisms tested against composites containing DMAHDM compared to control composites or composites without DMAHDM, confirming its antibacterial potency. One study reported increased antibacterial activity (decreasing CFU counts) with an increase in the mass fraction of DMAHDM [36]. Adding MPC to the DMAHDM composite had a synergistic antibacterial effect that decreased CFU counts much more than using either biomaterial alone as observed by

Reference	Biomaterial Combinations Used and Comparison Group/s	Microorganisms Tested	Time Points Assessed	Methodology Used to Assess Antibacterial Effectiveness	Results Summary
[35] Bhadila et al., 2020 [2]	(1) 20% NACP (2) 3% DMAHDM + 20% NACP Comparison group: (3) Heliomolar commercial composite	*S. mutans* [1][2][20][34][44]	2 days	Polysaccharide production	(1) had much less polysaccharide production than (3) and (4 The lowest production of polysaccharides from biofilms was caused by (2) [37][40][41][42][43]
				Live/dead assay	(2) had primarily dead bacteria compared to (1) and (3) which were primarily covered by live bacteria. [43]
				Production of Lactic Acid	(2) caused lowest production of lactic acid production from biofilms than (1) and (3)
				CFU counts	(2) showed a CFU reduction of 3–4 logs less than (1) and (3).
Zhou et al., 2020 [47] [37] [42]	(1) 30% NACP (2) 3% DMAHDM (3) 30% NACP + 3% DMAHDM Comparison groups: (4) 0% NACP + 0% DMAHDM (5) Heliomolar commercial composite	*S. mutans* *L. acidophilus* *C. albicans* Multi-species [20][44]	2 days	Live/dead assay [37][42]	(2) had substantial dead bacteria for all species tested while (1), (4) and (5) were covered with live bacteria.
				Metabolic assay (MTT)	(2) reduced the metabolic activity of biofilms significantly
				Production of Lactic Acid [40][41][42][43]	(1), (4) and (5) showed higher lactic acid production from *S. mutans* and polymicrobial biofilms while (2) and (3) inhibited. All materials produced lower levels of lactic acid from *L. acidophilus* and *C. albicans* [35]

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de production for all biofilms compared to NACP composites, which had similar polysaccharide production to commercial control composites [35][39][44]. MPC alone also significantly reduced polysaccharide production, however, a combination of DMAHDM + MPC displayed a synergistic effect exhibiting the least amount of polysaccharides for all types of biofilms tested [40][43]. Furthermore,

Reference	Biomaterial Combinations Used and Comparison Group/s	Microorganisms Tested	Time Points Assessed	Methodology Used to Assess Antibacterial Effectiveness	Results Summary
[41]				CFU counts [41]	(2) and (3) greatly reduced *S. mutans* and *C. albicans* CFU levels by 5 and 3 logs respectively
		[39]		Polysaccharide production	(2) and (3) inhibited production of extracellular matrix from the bacterial associated with root caries

5.5. Remineralization Results

There were five studies that assessed the remineralization potential of incorporating bioactive materials with DMAHDM (Table 4). All five studies incorporated NACP as a remineralizing agent, while one study also incorporated MPC, AgNPs and PAMAM to assess its remineralizing potential.

Table 4. Remineralization results.

Reference	Biomaterial Combinations and Comparison Group/s	Time Points Assessed	Methodology Used to Assess Remineralization	Results Summary
Zhang et al., 2016 [20]	(1) 20% NACP with QAM CL16 [a] Comparison group: (2) 20% NACP	1, 3, 7, 14, 21 and 28 days	Release of Ca and P ions	(1) released lower levels of Ca and P ions than (2)
Xiao et al., 2017 [23]	(1) 0.12% AgNPs + 3% MPC + 3% DMAHDM + 30% NACP (2) 0.12% AgNPs + 3% MPC + 3% DMAHDM + 30% NACP + PAMAM Comparison groups: (3) Demineralized root dentine specimen (4) Demineralized root dentine specimen + PAMAM	1, 3, 5, 7, 10, 14 and 21 days	Concentration of Ca and P ions	(1) and (2) demonstrated greater concentrations of Ca and P concentrations than PAMAM and control groups
			Acid neutralization	(1) and (2) had greater acid neutralization than PAMAM and comparison groups.
			Dentine hardness	(2) had the greatest dentine hardness, remineralization and mineral growth.
			SEMexamination	(2) had the greatest remineralization and mineral growth.

Reference	Biomaterial Combinations and Comparison Group/s	Time Points Assessed	Methodology Used to Assess Remineralization	Results Summary
Al-Dulaijan et al., 2018 [1]	(1) 20% NACP + 3% DMAHDM (2) 20% NACP Comparison group: (3) Heliomolar commercial composite	1, 3, 5, 7, 14, 21, 28, 35, 42, 49, 56, 63 and 70 days	Concentration of Ca and P ions	No differences in concentrations of Ca and P ions, their recharge and re-release between (1) and (2) groups
		1, 2, 3, 5, 9, 11 and 14 days	Recharge and rerelease of Ca and P	Specimens could release the ions for 42 days after one charge
Bhadila et al., 2020 [2]	(1) 20% NACP (2) 3% DMAHDM + 20% NACP Comparison group: (3) Heliomolar commercial composite	1, 3, 5, 7, 14, 21, 28, 35, 42, 49, 56, 63 and 70 days	Release of Ca and P ions	No significant difference in Ca and P ion release between (1) and (2)
		1, 3, 5, 7, 9 and 14 days	Recharge and rerelease of Ca and P	Both composites showed increasing ion concentration with time, and release continued after each recharge.
Zhou et al., 2020 [47]	(1) 30% NACP (2) 3% DMAHDM (3) 30% NACP + 3% DMAHDM Comparison groups: (4) 0% NACP+ 0% DMAHDM (5) Heliomolar commercial composite	1, 3, 7, 14, 21, 28, 35, 42, 49, 54, 63 and 70 days	Release of Ca and P ions	No difference in release of Ca and P ions between (1) and (3) Lower pH increased ion release
			Dentine hardness [20]	(3) caused the highest dentine hardness, which was twice

total time release in hree later studies, which found no significant difference between the NACP and DMAHDM + NACP composites [1][2][44]. Xiao et al. (2017) reported that DMAHDM + MPC + NACP + AgNPs and DMAHDM + MPC + NACP + AgNPs + PAMAM had higher calcium and phosphate concentrations than other control groups [23]. Two articles assessed ion recharge and rerelease and Ca and P ions. Both studies reported that NACP and DMAHDM + NACP continuously released ions after being recharged with no significant differences between them [1][2].

5.5.2. Other Results

Two studies evaluated dentine hardness at the dentine-restoration interface. Xiao et al. (2017) concluded that DMAHDM + MPC + NACP + AgNPs + PAMAM had the greatest dentine hardness, remineralization and mineral growth. Zhou et al. (2020) found that dentine hardness in DMAHDM + NACP group was more than double that of the control groups [23]. Xiao et al. (2017) further analyzed acid neutralization and a scanning electron microscopic examination (Table 4). It was reported that DMAHDM + MPC + NACP + AgNPs and DMAHDM + MPC + NACP + AgNPs + PAMAM had greater acid neutralization than PAMAM alone or other control groups [23].

6. Discussion

The aim of this systematic review was to report the current combinations of the DMAHDM composite and to assess the synergistic effects on the prevention of secondary caries. The findings from the studies included indicate that incorporating additional biomaterials with DMAHDM produces a positive synergistic effect on the prevention of secondary caries through antibacterial and remineralizing capabilities.

Regardless of the type of biomaterial combination added to the composite, all studies considered in this review reported a strong antibacterial efficacy of DMAHDM alone, with one study observing increased potency as the mass fraction increased [36]. It is suggested that the mechanism of action of QAMs is through contact inhibition leading to cell death [40][41]. However, it is essential that salivary protein adsorption occurs on the composite surface for bacterial adhesion to follow, and this pellicle separating the resin surface from the biofilm could decrease QAM's antibacterial efficacy [3][23][40].

MPC is a biocompatible polymer that was shown to have a synergistic mechanism of action when incorporated with DMAHDM compared to either agent alone [35][37][40][41][42][43]. Due to its hydrophilic surface, MPC can inhibit bacterial adhesion and decrease protein adsorption [35][40][46], thus, forming direct contact of the resin surface with the overlaying biofilms and enhancing the contact-killing mechanism of DMAHDM [35][40].

The literature reported DMAHDM + MPC having less biofilms with mostly compromised cell membranes, decreased CFU counts, the least metabolic activity, stronger killing efficacy, least lactic acid production, decreased protein adsorption and maintained a pH above 6.5 [41]. However, Zhang et al. (2017) observed live bacteria on the MPC + DMAHDM composite despite a reduction in lactic acid production from biofilm and total microorganism CFU counts [42]. This further emphasizes the need for more research into the long-term effectiveness of these novel composites on the prevention of secondary caries in orally healthy individuals.

Several articles also reported on the efficacy of novel composites against root caries pathogens [23][35][39][40][43][44]. Composites containing DMAHDM and MPC produced significantly lower levels of lactic acid from *S. mutans* and polymicrobial biofilms [1][2][20][34][44]. Lactic acid is a known byproduct of caries-causing bacteria, and coupled with their aciduric properties, allows them to survive in low pH conditions [44]. By reducing the amount of lactic acid, root dentine demineralization at the restoration margins may be reduced [44], which can improve the longevity of these restorations.

Combining MPC with DMAHDM was effective in inhibiting extracellular matrix synthesis of root caries pathogens by having substantially reduced polysaccharide production [44]. Polysaccharides are a key component of the extracellular polymeric substances (EPS) that surrounds biofilms and protects pathogens from antibacterial agents. Reducing polysaccharide production reduces this protection and the virulence of these pathogens, potentially reducing caries and inhibiting local periodontitis [47].

AgNPs have been associated with many dental applications such as acrylic resins for dentures [48], endodontic irrigants and intracanal medications [49][50] and other restorative materials [15][51]. The antibacterial properties of AgNPs is hypothesized to be due to the release of silver ions to the bacterial environment where the nanoparticle promotes infiltration into the bacterial cell membranes and affects intracellular processes [38]. Furthermore, the incorporation of AgNPs at 0.12% concentration with MPC and DMAHDM composite was shown to have even greater synergistic mechanisms of action, with further reductions of CFU counts, metabolic activity, protein adsorption and polysaccharide production [35]. AgNPs are particularly effective in inhibiting *S. mutans* and *F. nucleatum*, and are also capable of long-distance bacterial killing [35]. Incorporating small particles sizes of AgNPs increases the surface area, thus achieving a strong antimicrobial function with a relatively low filler level of AgNPs without compromising the mechanical properties or aesthetics of the resin [35][38].

Despite the interdisciplinary role AgNP has in modern medicine, recent reviews have discussed the possible environmental and economic impacts of AgNPs that are synthesized either naturally (green synthesis) or chemically [52][53][54][55]. Moreover, development of AgNPs using the biological method of synthesis with the use of bacteria, fungi and plant extracts [52][53] have shown to be ecofriendlier, cost effective and energy efficient [56], with reports of greater antimicrobial activity against pathogenic bacteria [52]. All three studies that experimented with AgNPs in this review synthesized the nanoparticles through chemical reduction. As such, further research is required to examine the effects of incorporating biosynthesized AgNPs on the efficacy of DMAHDM nanocomposite.

While NACP did not exhibit any antibacterial effect and showed comparable results to commercial control composites, NACP was effective on remineralization of tooth structure and pH neutralization [1][2][3][20][34][35][36][39][41][44]. The addition of NACP enables the release of Ca and P ions to increase the pH during cariogenic challenges, thereby, preventing demineralization and facilitating remineralization [35] and thus, reducing the potential for secondary caries development. Recent studies found no significant difference between the NACP and DMAHDM + NACP composites, meaning that DMAHDM did not impact the release of ions and can therefore be incorporated at no disadvantage [1][2][35]. Furthermore, the addition of NACP in the composite resin allows for repeated recharges, facilitates ion rerelease and remineralization over a longer period of time [1]. DMAHDM maintained potent antibiofilm properties after 12 cycles of recharge and did not affect ion re-release concentrations [1][2]. The findings from Bhadila et al. (2020) indicate that the concentration of Ca and P ion releases from NACP and DMAHDM combinations surpassed the required levels for tooth remineralization [57]. These results are very promising for improving the longevity and prognosis of current restorative materials.

Xiao et al. (2017) reported synergistic remineralizing effects such as greater acid neutralization, dentine hardness and mineral growth when combining NACP and third generation PAMAM. PAMAM exhibits the ability to remineralize tooth lesions through its role as an excellent nucleation template whereby Ca and P ions are rapidly absorbed leading to remineralization [23][58][59]. PAMAM has also shown lasting dentine mineral regeneration when incorporated with recharged NACP after prolonged fluid exposure [60], which indicates successful long-term therapeutic effects in reducing demineralization and aiding in the reduction of secondary caries.

Xiao et al. (2017) suggested that the incorporation of MPC, AgNPs, MPC and PAMAM with DMAHDM yielded the maximum antibacterial and remineralization capacity. The addition of MPC and AgNPs with DMAHDM produced significant synergistic antibacterial effects, while NACP and PAMAM provided continuous ion release and combined remineralization mechanisms of action [23]. Therefore, this novel bioactive composite combination shows promising results that may adjunctively reduce the rate of secondary caries and increase the longevity of these restorations.

The findings of this systematic review suggest that incorporation of antibacterial and remineralizing biomaterials have the potential to aid in the prevention of secondary caries. However, caries is a complex multifactorial disease and other factors must be considered when determining the clinical success of composite resins. These include but are not limited to, quality of the restoration such as the presence of microgaps and patient caries risk including oral hygiene habits, salivary flow and composition, consumption of dietary sugars and exposure to fluoride [61].

The limitations of this review include medium risk of bias and in vitro conditions in all studies. Additionally, there were wide variations of comparison groups between the studies. This study only focused on antibacterial and remineralization properties and did not consider mechanical qualities during water-aging. The bacterial incubation period for samples tested for antibacterial efficacy were heterogeneous across the studies, ranging from two days in some studies to 185 days in one study. The differences in incubation protocols may have depended on the manufacturer's instructions, pH of biofilm culture medium and that different types of bacteria required different incubation times. Therefore, it is important to note that these variations were adjusted accordingly to target specific bacterial species for different studies.

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