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Review

Nanotechnology-based therapies for the prevention and treatment of *Streptococcus mutans*-derived dental caries

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ABSTRACT

Background: Dental caries results from long-term acid production when sugar is metabolized by a bacterial biofilm, resulting in a loss of calcium and phosphate from the enamel. *Streptococcus mutans* is a type of acid-producing bacteria and a virulent contributor to oral biofilms. Conventional treatment options, such as cefazolin and ampicillin, have significant levels of bacterial resistance. Other topical agents, such as fluoride, tend to be washed away by saliva, resulting in low therapeutic efficacy.
Highlight: This review aims to highlight the solubility issues that plague poorly water-soluble therapeutic agents, various novel polymeric, and lipid-based nanotechnology systems that aim to improve the retention of therapeutic agents in the oral cavity.
Conclusion: In this review, different formulation types demonstrated improved therapeutic outcomes by enhancing drug solubility, promoting penetration into the deep layers of the biofilm, facilitating prolonged residence time in the buccal cavity, and reducing the emergence of drug-resistant phenotypes. These formulations have a strong potential to give new life to therapeutic agents that have limited physicochemical characteristics.

Contents

Abbreviations: AgNPs, silver nanoparticles; gtf, glycosyltransferase; GA-AgNPs, gum arabic-silver nanoparticles; LED, light emitting diode; PDT, photodynamic therapy; TMB, (3,3′,5,5′-tetramethylbenzidine).

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1. Introduction

Dental caries, commonly known as tooth decay or cavities, is one of the most common, preventable, and non-life-threatening diseases. Approximately 80% of adults are expected to experience dental caries by the age of 34 years [1]. In addition, 10% of children and 26% of adults have experienced untreated tooth decay, leading to abscesses or even death [1]. In the United States, approximately 124 billion dollars are spent annually on oral healthcare, and approximately 2.3 billion people have untreated tooth decay worldwide [2].

Dental caries results from long-term acid production, which weakens tooth enamel. Acid production occurs when sugar is metabolized by a bacterial biofilm, resulting in the loss of calcium and phosphate from the enamel. The process of calcium and phosphate loss is called demineralization. Repeated demineralization over a prolonged period leads to the formation of dental caries [3,4]. The bacterial biofilm-associated with dental caries forms through a series of steps initiated by attachment of the microorganism, which absorbs reversibly to the surface of the enamel. Thereafter, irreversible attachment occurs with the consequent production of an extracellular polymeric substance (EPS) matrix by the bacterial microcolonies, resulting in the formation of a three-dimensional biofilm [5]. *Streptococcus mutans* is one of many different types of bacteria found in the oral cavity and the most abundant and virulent contributor to the oral microbiome. Moreover, *S. mutans* has been implicated in several cases of dental caries [6].

Fluoride is the most common approach used to prevent dental caries formation. However, some bacterial strains, including *S. mutans*, have been reported to be resistant to fluoride [7]. In addition, excessive fluoride use, also known as fluorosis, in children who are still developing permanent teeth can result in faint white streaks on the teeth. Chlorhexidine is another effective antimicrobial agent for the management of dental caries. However, its use is delayed by the tendency to discolor the teeth [8–10]. Topical agents tend to be washed away by saliva, which reduces their retention, resulting in low therapeutic efficacy. At present, *S. mutans* is susceptible to cefazolin, ampicillin, and cefotaxime; however, significant resistance levels have been observed with other antimicrobials, including penicillin, clindamycin, erythromycin, and amoxicillin [11]. This review discusses the various novel nanotechnology-based approaches used to manage and treat *S. mutans* biofilms and the potential benefits of such systems. We will begin with a brief discussion of the pathophysiology of the disease with emphasis on the formation of biofilms and proceed to further elaborate on the various nanotechnology-based approaches available.

2. Background

2.1. Pathophysiology and the formation of biofilms

The development process of dental caries can occur throughout life, both in primary and permanent dentitions and can damage the tooth crown and expose root surfaces later. As best described by Pitt et al., dental caries is a complex biofilm-mediated disease caused by frequent ingestion of fermentable carbohydrates (mainly free sugars), poor oral hygiene, and inadequate fluoride exposure [12]. Before the first tooth erupts, the oral cavity contains both good and bad flora. This bad flora can cause dental caries and adhere to the mucosal tissue before the first tooth appears [5,13]. If caution is not taken, the risk of dental caries can dramatically increase in children under the age of two.

A film of proteins and glycoproteins derived mainly from saliva covers the tooth surfaces. The content of this conditioning film, known as an acquired pellicle, gradually becomes reinforced with bacterial components and their products, gingival crevicular fluid, blood, and food [12]. The process of dental caries is initiated by demineralization below the enamel surface by organic acids produced by biofilm bacteria from dietary sugar metabolism [14,15]. Lactic acid is the key organic acid generated from dietary sugars by dental biofilm microorganisms, including *S. mutans* [13]. Such acid build-up in the biofilm fluid generates low pH, leading to partial dissolution of calcium and phosphate (demineralization) of the surface layer of the tooth. A progressive increase in porosity and deeper diffusion of acids into the tooth results in subsurface demineralization. Neutralization of biofilm fluid pH can occur by swallowing and salivary dilution, and the presence of fluoride can inhibit the demineralization of the surface layer. In addition, the presence of calcium, phosphate, and fluoride ions promoted remineralization [16]. However, further progression of the caries process may lead to cavitation in the enamel and compromised dental pulp, culminating in either a root canal treatment or tooth extraction [12]. The risk of developing dental caries depends on age, tooth crowding, diet, morphology, and frequency of fluoride exposure [17–19].

The formation of a bacterial biofilm is a complex process that proceeds through a series of steps. First, the microorganism attaches reversibly to the surface. Thereafter, irreversible attachment occurs, followed by proliferation and production of the extracellular polymeric substance matrix, which encases the bacteria. In the third step, a three-dimensional biofilm was formed [5,13]. The final step involves maturation, detachment, and dispersal of the biofilm. The process of dental caries formation is fueled by the presence of microorganisms with traits that are relevant to caries. Studies of caries lesions found higher proportions and incidence of *S. mutans, Streptococcus sobrinus,* and *lactobacilli*. In addition, links between caries and other acid-producing and acid-tolerant bacteria, including a range of *Bifidobacterium* spp., *Actinomyces* spp., and *Propionibacterium* spp., have been reported [12]. However, the relative abundance and virulence of *S. mutans* is due to its ability to produce large amounts of EPS matrix and its adaptation to harsh conditions. The EPS matrix reduces the penetration of antibiotics, resulting in drug-resistant phenotypes. An effective antimicrobial agent must be retained in the oral cavity without saliva washing away to combat antimicrobial permeability and resistance issues. It

should be non-toxic and mucoadhesive to allow retention in the oral cavity. In addition, it should be capable of penetrating both the polysaccharide matrix, which is negatively charged, and penetrate the three-dimensional biofilm structure. Specifically, antimicrobial agents formulated with cationic polymers would provide positive charges that could facilitate electrostatic interactions with the negatively charged polysaccharide matrix and increased penetration [20].

Nanotechnology-based systems, such as nanoparticles, liposomes, and emulsions, are attractive due to their ability to entrap poorly water-soluble therapeutics agents. For example, nanoparticles can be biocompatible, mucoadhesive, and non-toxic. Some can also be formulated to have antimicrobial cationic polymers, which effectively penetrate the negatively charged EPS matrix. This review article discusses nanotechnology-based therapies to combat *S. mutans* bacterial biofilms.

2.2. Gaps in the current research

The daily saliva production from an average adult ranges from approximately 500 mL to 1500 mL [21]. The unstimulated saliva flow rate was between 0.12 and 0.16 mL/min [22,23]. Under stimulated conditions, the flow rate of saliva can increase to a maximum of 7 mL/min**.** As a result of a large amount of saliva and the variable flow rate, most treatments rapidly washed away, leading to a short residence time. The novel antibacterial therapies outlined below have increased viscosity which help to improve the residence time and efficacy. However, they do not necessarily eradicate biofilms. There is also a need to continue to improve the residence time and efficacy, and address the issues associated with antibiotic resistance.

2.3. Characteristics of novel nanotechnology-based drug delivery systems

Nanotechnology-based drug delivery systems are a rapidly growing field due to their potential to carry larger payloads of poorly water-soluble therapeutic agents. This feature allows us to investigate drugs that would otherwise have limited value and use because of their poor water solubility. We have a wide range of nanotechnology-based drug delivery systems at our disposal, such as polymeric nanoparticles, liposomes, dendrimers, and emulsions. The average hydrodynamic diameter or particle size varies but is often sized between 100 and to 1000 nm. These systems are commonly formulated with biocompatible and biodegradable polymers and lipids. In addition, these polymers and lipids tend to be more viscous. Therefore, they are more effective for retention in the oral cavity, thus improving efficacy. Some systems can be modified to provide either controlled or immediate drug release. Lastly, many nanotechnology-based systems can be surface-modified with cationic polymers and surfactants, facilitating electrostatic interactions with negatively charged epithelial cells. This improved retention in the oral cavity and improved efficacy. In summary, it is a combination of physicochemical characteristics that enhance therapeutic efficacy.

Below is an overview of the benefits and applications of various types of nanotechnology-based systems that have been used to treat *S. mutans* biofilms (Table 1).

3. Novel treatments: antibacterial approaches

3.1. Polyethylenimine nanoparticles

Dental caries can be problematic in patients who use orthodontic braces. New carious lesions were found in 45.8% of patients

Table 1
A summary of the various nanotechnology-based formulations used to treat dental caries.

Formulation	Composition	Novel Characteristic	Ref.
PEI Nanoparticles	Polyethylenimine (PEI)	PEI is a cationic insoluble polymer with a longer duration of stability.	[24]
Cyclodextran Nanocomplex	α-cyclodextran, hydroxypropyl-β-cyclodextrin, or Y-cyclodextran	Ability to form non-covalent complexes with both water soluble and poorly/water-soluble compounds.	[28,32]
Dextran coated iron oxide nanoparticles	Various molecular weights of dextran and iron oxide	Iron oxide has high catalytic activity and is anti-microbial agent.	[35]
Nanoemulsion	Essential oils and tween 20 or Cremophor EL	Can potentially be used as a mouthwash. Capable of entrapping hydrophobic compounds and has a longer shelf-life.	[38,40,41]
Liposomes	Various lipids, soy lecithin	Can entrap hydrophobic and hydrophilic compounds. Longer shelf-life.	[43,44]
pH-activated nanoparticles	A pH activated polymer p(DMAEMA-co-BMA-co-PAA) and the cationic polymer poly(dimethylaminoethyl methacrylate) (p(DMAEMA))	Capable of loading poorly-water soluble compounds, provides pH-controlled drug release.	[49]
Silver nanoparticles	Silver, gum arabic	Has antioxidant and antimicrobial properties. Inhibits quorum sensing.	[55,65]
PLGA Nanoparticles	Poly-lactic-co-glycolic and acid. Can contain: PEG, cationic, or anionic surfactants	Can entrap poorly water-soluble compounds, longer shelf life.	[69,73]
PAMAM Dendrimers	Polyamidoamine and can be surface functionalized	Can improve the solubility of poorly water-soluble therapeutic agents.	[33,76]
Hydrogels	Chitosan	Can entrap hydrophilic compounds but has a higher viscosity which can increase the residence time. Chitosan can provide pH-controlled release.	[79]

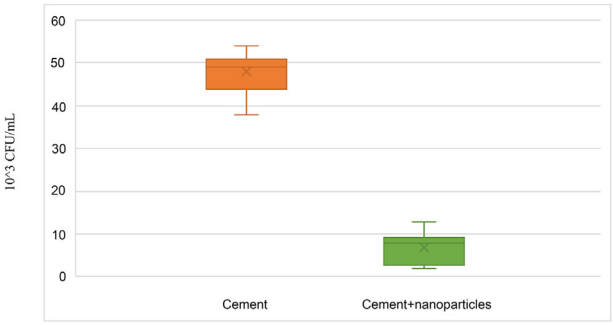

Fig. 1. "Neobond incorporating 1% quaternary ammonium polyethylenimine nanoparticles reduces bacterial viable counts of *S. mutans* adjacent to bonded brackets." This figure was reprinted with permission from Sharon, E. et al., 2018 [24].

using orthodontic braces [24,25]. Factors that can contribute to the increased rate of dental caries include patient age, poor oral hygiene, and duration of treatment [24]. Braces-induced lesions can be reduced using polymers, and two main types of polymers can be used to produce nanoparticles. The first type is soluble polymers such as chitosan, characterized as having a shorter duration of efficacy due to their increased solubility. Alternatively, insoluble polymers can be used to compensate for this problem. Insoluble polymers, such as polyethylenimine, have the potential to retain an antimicrobial effect for a longer time and are likely to have a longer duration of stability. A previous study incorporated quaternary ammonium into polyethylenimine nanoparticles to create antibacterial nanoparticles. Quaternary ammonium is a cationic surfactant that has biocidal properties that interfere with and lyse the cell membrane [26]. In this study, sterilized crowns were coated with quaternary ammonium polyethylenimine nanoparticles, and bonded brackets were placed on the crown [24]. The crowns were subsequently immersed in *S. mutans* and incubated at 37 °C for 48 h. Antibacterial activity was evaluated using the crystal violet assay. The nanoparticle-treated brackets resulted in a 66% reduction in bacterial mass as compared to the non-treated brackets [24]. Thereafter, the bacterial count was determined using a similarly designed method. The nanoparticle-treated cemented brackets saw a significant reduction in the viable bacterial counts compared to the cemented non-treated brackets (Fig. 1). It can be concluded that these quaternary ammonium polyethylene nanoparticles have good antimicrobial activity and a potential for longer-lasting effects due to their insolubility.

3.2. Nano-complexes

Covalently conjugated polymer-drug therapies can improve the solubility and stability of therapeutic agents. However, there are often multiple potential conjugation sites for both polymers and therapeutic agents. When a therapeutic agent is a protein, it can negatively impact its efficacy. For example, in many cases, the polyethylene glycol (PEG) polymer in a PEG-protein conjugate can conjugate to and interfere with the recognition site on the protein, thus decreasing the efficacy [27]. To combat this, non-covalent complexes can be formed using CD polymers. Cyclodextran forms an inclusion complex with a therapeutic agent, minimizing the polymer-drug interaction. Cyclodextran polymers also have the unique ability to encapsulate both hydrophilic and hydrophobic therapeutic agents [28]. One particular study took advantage of these features and created a Rose Bengal α-cyclodextran nanoparticle [28]. Rose Bengal (4,5,6,7-tetrachloro-2′,4′,5′,7′-tetraiodofluorescein) is a hydrophilic photosensitizer with poor intracellular uptake. Cyclodextran's ability to form a non-covalent complex is critical for improving the efficacy of this system. In addition, planktonic *S. mutans* bacterial suspensions were cultured for 18 h before use and then treated with Rose Bengal loaded α-cyclodextran nanoparticles for 24 h. Planktonic suspensions were subjected to photodynamic therapy (PDT) with brief irradiation periods (60 s). In PDT, microorganisms are treated with a photosensitizing agent, then exposed to lasers at low power to kill the bacteria [29–31]. Traditionally, PDT uses lasers as the light source. However, this study utilized a blue light-emitting diode (LED) as the light source. Blue LEDs are widely available in dental practice for whitening teeth. Compared to lasers, LEDs have a wider emission spectrum, are cheaper, and easier to use. There was no indication of viable bacteria in cultures treated with 62 nM Rose Bengal-loaded α-cyclodextran nanoparticles. At similar concentrations of pure Rose Bengal, only partial antibacterial activity was observed. It took 10× the amount of pure Rose Bengal to produce the same effect as 62 nM Rose Bengal-loaded α-cyclodextran nanoparticles.

Cyclodextran can also be used to prevent hydrolysis of the therapeutic agent from being hydrolyzed. For example, titanium tetrafluoride was previously shown to protect against dental erosion and prevent biofilm formation; however, its applicability is limited due to its extensive hydrolysis [32]. Therefore, 1% titanium tetrafluoride was loaded into hydroxypropyl-β-cyclodextrin or γ-cyclodextran complexes prepared for 12 or 72 h period [33,34]. Bovine enamel blocks were pretreated with the samples for 1 min and then washed. The inoculum was added and incubated for 48 h to ensure a prevalent biofilm. The cyclodextran nanoparticles without titanium tetrafluoride showed no bactericidal activity. All the complexes with titanium tetrafluoride yielded a minimum bactericidal concentration (MBC) of 0.25%, whereas pure 1% titanium tetrafluoride showed an MBC of 0.13%. However, a cell viability assay using mammalian fibroblast cells indicated that 1% titanium tetrafluoride reduced the viability to 65.6% after 24 h, indicating significant cytotoxic effects. The 72 h Hydroxypropyl-β-cyclodextrin and γ-cyclodextran complexes containing 1% titanium tetrafluoride showed the least cytotoxicity at 75.7% and 74%, respectively. Similarly, both formulations demonstrated bactericidal activity.

3.3. Iron oxide nanoparticles

Iron oxide nanoparticles have high catalytic activity, which disrupt the bacterial biofilm (i.e., plaque) present on the teeth. Iron oxide nanoparticles lack clinical relevance due to their poor stability at physiological pH. This issue can be addressed by coating iron oxide nanoparticles with polymers. For example, Naha et al. improved the stability and maintained the therapeutic efficacy of iron oxide nanoparticles by coating them with dextran [35]. Dextran, a Food and Drug Administration (FDA)-approved polymer,

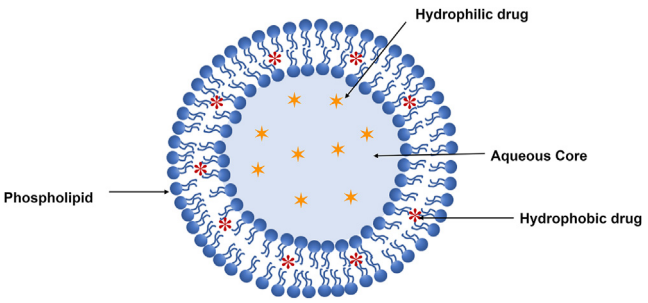

Fig. 2. Schematic representation of a liposome encapsulating a hydrophilic and hydrophobic drug.

is available in a range of molecular weights. In this study, molecular weights between 1.5 and 40 kDa were used. The hydrodynamic diameter ranged from 35 to 62 nm for all dextran-coated iron oxide nanoparticles. The lowest molecular weight (35 ± 0.2 nm was observed for the 10 kDa dextran-coated iron oxide nanoparticles. These nanoparticles had a corresponding zeta potential of −18 ± 0.2 nm. The peroxidase-like catalytic activity of iron oxide was quantified using a colorimetric TMB (3,3′,5,5′-tetramethylbenzidine) assay at pH 4.5, 5.5, and 6.5. Higher catalytic activity was observed at a lower pH of 4.5 and with the 10 kDa dextran-coated iron oxide nanoparticles. It was determined that iron oxide, but not dextran, contributed to catalytic activity. The authors proposed that this was most likely due to the smaller hydrodynamic diameter, which resulted in a larger surface area. Bactericidal assays showed the greatest reduction of 6-log colony-forming units (CFUs) when the biofilm was treated with iron oxide nanoparticles coated with 1.5, 5, or 10 kDa dextran. Iron oxide nanoparticles coated with higher molecular weight dextrans (25 and 40 kDa) were not able to reduce the total number of CFUs and the nanoparticles made with lower molecular weight dextrans. Iron oxide nanoparticles coated with 10, 25, or 40 kDa dextran all showed a lower dry weight bacterial biomass after the treatment than the hydrogen peroxide treatment (control). Iron oxide nanoparticles coated with 10 kDa dextran demonstrated bacterial activity and high biofilm mass disruption. As a result, this study concluded that the only successful formulation was iron oxide nanoparticles coated with 10 kDa dextran.

3.4. Nanoemulsions

Emulsions are thermodynamically unstable systems consisting of oil and water phases brought together using surfactants and an energy source such as microfluidization. In oil-in-water emulsions, the oil is dispersed as droplets in the water phase. They commonly result in diameters ranging from 10 to 400 nm. A wide range of bactericidal and disinfectants can be loaded into emulsion formulations. In contrast to most polymeric nanoparticles, emulsions can also be shelf-stable for a couple of years [36,37]. Cho et al. prepared nanoemulsions containing *Curcuma xanthorrhiza* oil [38]. *C. xanthorrhiza* is derived from turmeric plants and has excellent antimicrobial activity. *C. xanthorrhiza* is very hydrophobic and is often solubilized using dimethyl sulfoxide (DMSO), which can be toxic to young children. Therefore, a nanoemulsion was prepared using *C. xanthorrhiza*, water, and Tween 80. The hydrodynamic diameter was determined to be 62.1 nm with a polydispersity index of 0.17 [38]. *S. mutans* biofilms grown on sterile hydroxyapatite disks were treated with either the *C. xanthorrhiza* nanoemulsion, distilled water, or Listerine® for 1 min every 4 h for 2 days. The viable bacteria counts for the nanoemulsion, distilled water, and Listerine® were determined to be: 5.72 $\log_{10}$ CFU/mL, 7.83 $\log_{10}$ CFU/mL, and 7.52 $\log_{10}$ CFU/mL, respectively [38]. These results demonstrate a strong antimicrobial effect and highlight their potential use in the treatment of dental caries.

Another study involved the formulation of triclosan, a bacteriostatic agent, in an emulsion. The use of triclosan is controversial; studies have shown that prolonged exposure to triclosan can increase the risk of microbial resistance [2]. In addition, the FDA banned the use of triclosan in over-the-counter products partially because of the manufacturers' inability to show efficacy [39]. Despite these issues, one study demonstrated the reversal of triclosan resistance by loading triclosan into cationic chitosan nanocapsules [40]. In a similar study, Franklyne et al. formulated a triclosan-containing essential oil-based emulsion [41]. The principle of this formulation is based on the observation that essential oils and essential oil-containing mouthwashes can enhance the activity of antibacterial agents. Emulsions were prepared with either cinnamon oil, clove oil, peppermint oil, or eugenol oil, with Tween 20 or Cremophor EL (peppermint oil emulsion) as the surfactant. All of the emulsions had hydrodynamic diameters between 10 and 19.3 nm. The highest hydrodynamic diameter was observed for the emulsion containing peppermint oil. Of all the drug-free emulsions tested, the eugenol oil emulsion had the lowest minimum inhibitory concentration (MIC)/minimum bactericidal concentration (MBC) values of 3.125 μL/mL compared to clove, cinnamon, and peppermint oil emulsions, each having an MIC of 25 μL/mL. Similarly, compared to non-formulated triclosan having a MIC/MBC of 0.125 μg/mL/125 μg/mL and triclosan-containing emulsions (clove oil, 0.06 μg/mL/12.5 μg/mL; cinnamon oil, 0.06 μg/mL/12.5 μg/mL; and peppermint oil, 0.125 μg/mL/12.5 μg/mL), triclosan-loaded eugenol oil emulsion had a significantly lower MIC/MBC (0.015 μg/mL/1.6 μg/mL) [41]. In conclusion, the triclosan-loaded eugenol oil emulsion showed the highest efficacy compared to all other formulations tested in this study. Furthermore, it demonstrated that a eugenol oil-containing emulsion is potentially able to combat triclosan resistance issues.

3.5. Liposomes

Liposomes can entrap hydrophobic drugs within the lipid bilayer and hydrophilic drugs within the aqueous core (Fig. 2). They are biocompatible, can improve drug solubility and release drugs slowly over time. Because of these improved characteristics, liposomes can enhance therapeutic efficacy. As mentioned in the previous section, essential oils have been reported to have good antimicrobial activity and with antioxidant properties [42]. However, they tend to have poor water solubility and high volatility and are susceptible to degradation [43]. To address these limitations, a study formulated liposomes with phosphatidylcholine and either citral or *pompia* essential oil extract through self-assembly in water. Citral is an essential oil and one of the main components of *pompia* essential oil extract. The hydrodynamic diameter was determined using dynamic light scattering. The hydrodynamic diameter of the empty liposomes was 130 ± 9 nm [43]. Liposomes containing 12, 25, or 50 mg/mL extract had sizes of 110 ± 7 nm, 114 ± 8, and 117 ± 9 nm, respectively, whereas liposomes containing 12, 25, or 50 mg/mL citral were 105 ± 8, 99 ± 9, and 97 ± 7 nm, respectively. The formulations were determined to be polydisperse with a polydispersity index between 0.268 and 0.430, with the highest polydispersity index of 0.430 ± 0.097 observed with empty liposomes [43]. All liposomal formulations demonstrated a high loading efficiency, ranging between 86% and 91% [43].

The antibacterial activity was determined by measuring the diameter of the inhibition zone using 50 mg/mL extract-loaded or citral-loaded liposomes. The extract-loaded liposomes did not significantly inhibit *S. mutans* with an inhibition zone of less than 5 ± 1 mm. However, the citral-loaded liposomes significantly inhibited *S. mutans* by more than 3-fold in the zone of inhibition (16 ± 1 mm) [43]. An alternative formulation of citral in a dispersion composed of phospholipids showed a slightly lower inhibition diameter of 11 ± 1 mm [43]. This study concluded that citral-loaded liposomes were the optimal formulation. The authors proposed that these liposomes can be incorporated into a mouthwash for the treatment of dental caries in *S. mutans*.

A second study incorporated *Thymus capitatus* extract into liposomes, glycerosomes, and penetration enhancer-containing vesicles [44]. *Thymus* has also been reported to have significant antimicrobial and antioxidant properties. The liposomes were prepared by the self-assembly of soy lecithin in water, whereas glycerosomes and vesicles were prepared using glycerol or propylene glycol in water. Antioxidant activity was investigated in

keratinocytes by initial exposure to hydrogen peroxide, an oxidative agent, followed by estimation of cell viability (i.e., ~65%). Thereafter, the cells were treated with either thymus-extract-loaded liposomes, glycerosomes, or vesicles. Significantly, all three formulations increased cell viability from ~65% to 90%–100% compared to hydrogen peroxide and *T. capitatus* extract, respectively. This underscores their potent antioxidant activity. Next, antibacterial activity was determined by measuring the diameter of the incubation zone. The incubation zone diameters were as follows: 13 ± 4 mm for the liposomes, 11 ± 3 mm for the glycerosomes, and 11 ± 2 mm for the vesicles. Notably, increasing the amount of glycerol in the formulation (glycerosomes) had no effect on the inhibition zone diameter [44]. Furthermore, doubling the amount of propylene glycol in the vesicle formulation increased the incubation zone diameter from 7 ± 3 mm to 11 ± 2 mm. It was concluded that all these formulations had excellent antioxidant activity and inhibitory effect on *S. mutans*. The authors also proposed that these formulations could be incorporated into a mouthwash [44].

3.6. pH-activated nanoparticles

The pH of dental plaque is estimated to be 4.5 to 5.5 [45–48]. Maintaining this low pH in the oral cavity induces synthesis of the EPS matrix, which helps to maintain a prevalent biofilm. pH-activated nanoparticles have the potential to be loaded with high concentrations of poorly water-soluble antibacterial agents, be retained in the oral cavity for a longer time owing to their bioadhesive nature, and release them at an acidic pH, providing a high concentration of therapeutic agents directly at the target site. One study fabricated a pH-activated micelle-based nanoparticle loaded with the poorly water-soluble antibacterial agent, farnesol [49]. Farnesol has been shown to disrupt the synthesis of the EPS matrix, which is thought to damage the structural integrity of the biofilm [50,51]. The hydrophobic core of these pH-activated nanoparticles consisted of a pH-activated polymer, p(DMAEMA-co-BMA-co-PAA), and farnesol. The outer surface of the nanoparticle consisted of the cationic polymer poly(dimethylaminoethyl methacrylate) (PDMAEMA). When exposed to an acidic pH, the core polymers, polyacrylic acid (PAA) and (PDMAEMA), become positively charged, creating electrostatic repulsion due to the cationic surface polymers. The electrostatic repulsion destabilizes the nanoparticle and damages the structural integrity, which causes the release of farnesol.

These pH-activated nanoparticles exhibited higher drug loading capabilities of up to 20% of their weight with farnesol, far exceeding the results of a separate study that showed that a pluronic-based micelle can only load up to 1% of its weight with farnesol [52]. Furthermore, the corresponding loading efficiency of the farnesol pH-activated nanoparticles was greater than 90%. These farnesol-loaded pH-activated nanoparticles exhibited a pH-dependent release of farnesol, with approximately 75% of farnesol released within 12 h at pH 4.5, compared to the same amount of farnesol taking nearly 30 h was released at pH 7.2.

Furthermore, the farnesol pH-activated nanoparticles exhibited an 80% decrease in colony-forming units (CFU) per biofilm, whereas free farnesol resulted in only a 20% decrease in CFUs per biofilm. Similarly, the farnesol pH-activated nanoparticles exhibited a 2-fold increase in biofilm removal. In Sprague–Dawley rats orally inoculated with *S. mutans,* treatment of the rats with twice daily oral topical applications of the farnesol-loaded pH-activated nanoparticles resulted in a reduction in the number and severity of dental caries, as measured using a modified Keyes' scoring system. Conversely, free farnesol had no positive effects. In conclusion, farnesol-loaded pH-activated nanoparticles can weaken and destabilize biofilms.

3.7. Silver nanoparticles

Silver nanoparticles are metallic nanoparticles with many therapeutic applications, such as cancer treatment, skin burn treatment, and antiviral therapy. In addition, they possess antioxidant and antimicrobial properties. However, toxicity has been reported, particularly at higher concentrations. Toxicity is affected by several formulation factors, including particle size, surface chemistry, shape, ionic strength, and pH [53,54]. Examples of potential toxic side effects include liver and kidney damage.

In addition to their other therapeutic applications, silver nanoparticles have been reported to prevent the formation of *S. mutans*-associated dental caries by inhibiting quorum sensing [55–57]. Quorum sensing is a bacterial cell-to-cell communication process that involves the regulation of gene expression in response to fluctuations in cell population density [58,59]. Bacteria employ quorum sensing communication circuits to regulate various physiological activities such as virulence, competence, conjugation, antibiotic production, motility, sporulation, and biofilm formation. *S. mutans* utilizes QS to regulate biofilm formation. Quorum sensing in *S. mutans* involves signaling through a two-component signal transduction system mediated by a competence-stimulating peptide. Binding of a competence-stimulating peptide to the membrane-bound two-component histidine kinase receptor activates the kinase activity of the receptor. Such kinase activation results in phosphorylation of its cytoplasmic response regulator. The phosphorylated response regulator activates transcription of genes in response to the signaling peptide [60–62].

In another study, the authors synthesized silver nanoparticles (AgNPs) using gum arabic (GA), which was derived from the tree *Acacia senegal* (L) wild, where gum arabic served as a stabilizer to promote capping in the silver nanoparticles, as well as improving the biocompatibility of silver nanoparticles [55]. The inhibitory effect of GA–AgNPs was determined using the agar diffusion method. Briefly, *S. mutans* were plated on LB agar plates, followed by treatment with 25, 50, 100, and 200 μg/mL of GA-AgNPs for 24 h at 37 °C and the diameter of the incubation zones was recorded. A 0.1% aliquot of DMSO served as a negative control. The smallest incubation zone diameter was 14.05 ± 0.7 nm, as seen when *S. mutans* were treated with 25 μg/mL nanoparticles (Table 2). The largest incubation zone diameter was 18.3 ± 0.5 nm with 200 μg/mL nanoparticles [55]. The minimum inhibitory concentration was 10 μg/mL. The mechanism of action of the GA-AgNPs is believed to be via inhibition of gyrase A gene expression, which plays a functional protective role in *S. mutans* from environmental stress and antibiotics [63]. In addition, GA-AgNPs were shown to inhibit glycosyltransferase gene expression (i.e., gtfB, gtfC, gtfD, and gdpB) [55]. Glycosyltransferase has been shown to significantly contribute to plaque formation by *S. mutans* [64]. The authors concluded that the AgNPs were effectively prevented *S. mutans* dental caries and proposed their formulation or potential incorporation in oral hygiene products such as mouthwash or toothpaste.

Table 2
The antibacterial effect of gum arabic-silver nanoparticles. Adapted from Ref. [55].

Concentration (μg/mL)	Diameter of the incubation zone (nm)
25	14.1 ± 0.7
50	15.5 ± 0.8
100	16.3 ± 1.0
200	18.3 ± 0.5

A second study involved the fabrication of AgNPs using the fruit extract of olives, where the extract functioned as a reducing agent to facilitate the chemical reduction of silver nitrate ($AgNO_3$), resulting in the formation of silver nanoparticles [65]. Antibacterial activity was determined by measuring the diameter of the inhibition zones, which showed dose-dependent inhibitory effects of 25 μl/mL (14 mm), 50 μl/mL (19 mm), 75 μl/mL (20 mm), and 100 μl/mL (22 mm). Similarly, inhibition of biofilm (antibiofilm activity), evaluated using a crystal violet assay, followed a dose-dependent response at concentrations of 25 μl/mL (10.55%), 50 μl/mL (24.84%), 75 μl/mL (35.71%), and 100 μl/mL (54.23%) [65]. The authors proposed that these AgNPs likely inhibited bacteria and did not evaluate the inhibition of these enzymes. The authors concluded that silver nanoparticles demonstrated excellent antibacterial activity.

4. Remineralizing approaches

Remineralization can occur naturally when calcium and phosphate ions found in saliva are deposited in the voids of the demineralized structure. This process helps counteract the effects of demineralization. Remineralization results in a net gain of the ions [66]. However, natural remineralization through saliva is a slow process and is inadequate for facilitating remineralization efforts [66]. Fluoride use is used to promote the remineralization process; however, several studies have reported a plateau or even an increase in the number of dental caries despite fluoride use [66–68]. Therefore, it is important to investigate new remineralizing formulations to facilitate these efforts. Several remineralizing novel formulations are discussed in the following paragraphs. An ideal remineralization material can diffuse into the subsurface and deliver calcium and phosphate into the subsurface, functioning in an acidic pH, and boosting the remineralizing properties of saliva. In addition to the above properties, most of the materials discussed below also inhibit acid production by *S. mutans* by reducing the bacterial population.

4.1. Poly(lactic-co-glycolic) acid (PLGA) nanoparticles

S. mutans is a key bacterium involved in lactic acid production, leading to tooth decay. One treatment strategy is to use anti-acidogenic agents, such as flavones. However, flavones are easily washed away by the saliva. Therefore, Sebelemetja et al. developed a flavone-loaded PLGA-PEG nanoparticle [69]. PLGA is a synthetic, biocompatible, and biodegradable polymer approved by the FDA. PLGA nanoparticles have a hydrophobic core and hydrophilic surface. To form the hydrophobic core, the PLGA polymer is typically dissolved in an organic solvent, such as methylene chloride [70]. The methylene chloride is later evaporated; however, this enables us to entrap larger quantities of poorly water-soluble therapeutic agents compared to other types of nanoparticles that are not compatible with such solvents. PLGA nanoparticles can also provide controlled drug release over an extended period. This controlled drug release can be achieved by modulating the ratio of lactic and glycolic acids in the formulation.

Flavone (5,6,8-trihydroxy-7-methoxy-2-(4-methoxyphenyl)-4H-chromen-4-one) was extracted from the South African plant, *Dodonaea viscosa* var. *angustifolia* [69]. Nanoparticles were prepared using the double emulsion method (w/o/w) using PLGA-PEG. The MIC of pure flavone extract in DMSO was determined to be 0.78 mg/mL. In contrast, the MIC of the control nanoparticles (which contained no drug) and flavone loaded PLGA-PEG nanoparticles were determined to be 6.25 and 1.56 mg/mL, respectively. Although pure flavone showed a lower MIC, it is not clinically relevant because of its low residence time in the oral cavity due to being washed away by saliva. The effects of the flavone extract and the flavone-loaded PLGA-PEG nanoparticles on biofilm formation were shown to reduce the total bacterial cell count by 98.7% and 92.4% after 24 h, respectively [69]. Conversely, the control nanoparticles without the drug inhibited biofilm formation by 35.5–59.1% [69]. The anti-acidogenic assay indicated that the flavone extract and the flavone-loaded nanoparticles significantly inhibited *S. mutans* acid production. This suggested that the efficacy is a function of both the ability of the formulation to increase the environmental pH and the antimicrobial properties of flavones.

Another study evaluated the antimicrobial activity of curcumin-loaded PLGA nanoparticles for the treatment of white spot lesions. White spot lesions are demineralized regions of tooth enamel. They are a common complication in patients with fixed orthodontic appliances, such as braces. Curcumin is a hydrophobic compound derived from *Curcuma longa.* Curcumin has anti-inflammatory and antimicrobial properties and can be used as a photosensitizer [71,72]. PLGA nanoparticles were prepared using an electrospraying method and loaded with 3%, 5%, 7%, or 10% weight of curcumin. The release of curcumin from the PLGA nanoparticles was biphasic, with 6 %–8% curcumin being released in 1 h and 67% released within 6 days [73]. When the nanoparticles were added to the orthodontic adhesive, the adhesive remnant index of the curcumin-loaded PLGA nanoparticles was not statistically different from that of the orthodontic adhesive (control). The adhesive remnant index was used to assess the extent to which the orthodontic brackets bonded to the interface. However, the shear bond strength of the 7% curcumin-loaded PLGA nanoparticles was 14.89 ± 3.26 compared to 27.33 ± 5.17 as seen with the orthodontic adhesive (control). Even though the shear bond strength was lower, in combination with the adhesive remnant index, the authors concluded that the orthodontic adhesive containing 7% curcumin-loaded nanoparticles could serve as an effective adhesive [73]. Next, enamel slab brackets were bonded using orthodontic adhesive (control) or 7% curcumin-loaded PLGA nanoparticles and orthodontic adhesive (treatment). *S. mutans* biofilms were grown on enamel slab bonded brackets, and the optical density was measured over the course of 180 days. After 15 and 60 days, there was a significant 64.7% and 43.3% decrease in optical density, respectively, in the orthodontic adhesive containing the nanoparticles as compared to the control orthodontic adhesive [73]. Next, the *S. mutans* biofilms grown on enamel slab bonded brackets

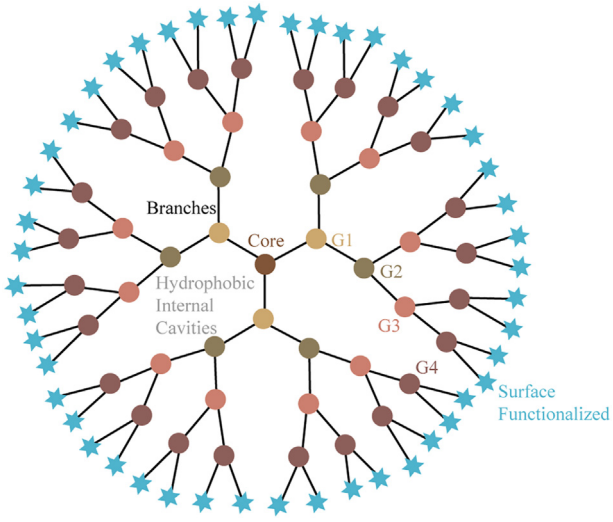

Fig. 3. Schematic representation of a 4th generation dendrimer. G1 - G4 stands for 1st – 4th generations.

were treated with curcumin-loaded PLGA nanoparticles and subjected to PDT therapy. After 15 and 60 days, there was a 94.1% and 69.6% reduction in optical density, respectively, as seen with the orthodontic adhesive containing the curcumin-loaded nanoparticles as compared to the control orthodontic adhesive. After 120 days, a 55.1% decrease in optical density was observed in the orthodontic adhesive containing the nanoparticle group as compared to those containing only the orthodontic adhesive. The authors concluded that curcumin-loaded PLGA nanoparticles added to orthodontic adhesive and treated with PDT have the potential to control the formation of *S. mutans* biofilms.

4.2. Dendrimers

Dendrimers are highly branched, regularly repeating structures with a hydrophobic core (Fig. 3). This core allows the entrapment of poorly water-soluble therapeutic agents. Dendrimers are not nanoparticles, but share similar characteristics, including hydrodynamic diameters, controlled drug release, improved drug loading, and incorporation of cationic polymers that possess both antimicrobial and mucoadhesive properties [74].

One study evaluated dendrimers for their potential to entrap and deliver apigenin [33]. Apigenin is a naturally derived flavonoid frequently found in many fruits and vegetables but has limited use because of its poor water solubility. Flavonoids have been shown to exhibit anti-inflammatory and anti-oxidative effects [75]. Specifically, apigenin disrupts the polysaccharides generated in dental biofilms [50].

In this study, third-and fourth-generation polyamidoamine (PAMAM) dendrimers were fabricated. The generation number refers to the number of branches in the dendrimer (Fig. 3). Both 3rd and 4th generation PAMAM dendrimers had similar apigenin loading capacities of 16.67% and 20.59%, respectively. In addition, within 12 h, 58% and 67% of apigenin was released from the 3rd and 4th generation dendrimers, respectively [33].

Sterilized hydroxyapatite slices were incubated for 24 h in one of the following solutions: phosphate buffer saline (PBS), 3rd or 4th generation PAMAM dendrimers without apigenin, or 3rd 4th generation apigenin-loaded PAMAM dendrimers. After 24 h, the slices were removed, dried, and incubated with *S. mutans* for 48 h. A live/dead viability assay was performed, which showed no effect on the biofilm viability by PBS, whereas third-and fourth-generation PAMAM dendrimers without apigenin produced dead/live ratio of 0.68 and 0.88, respectively. Notably, 3rd and 4th generation apigenin loaded PAMAM dendrimers significantly killed *S. mutans* with a dead/live ratios of 1.34 and 1.37, respectively (Fig. 4A–E) [33]. Both 3rd and 4th generation dendrimers demonstrated a strong ability to bind to human tooth samples, which is critical for prolonging the residence time and preventing removal by saliva. In a follow-up study, the apigenin-loaded PAMAM dendrimers effectively induced remineralization of demineralized enamel [76].

4.3. Hydrogels

A hydrogel is a polymeric network created through polymer cross-linking or swelling in water [77,78]. One of the most common types of polymers used in hydrogel formulations is chitosan. Chitosan is a biocompatible, biodegradable, non-toxic, and positively charged polymer. Chitosan can also act as a mucoadhesive agent to increase the residence time of tooth enamel. Chitosan is soluble in dilute acidic aqueous solutions, which allows for the entrapment of both hydrophilic therapeutic agents and peptides. Studies have demonstrated the practical application of chitosan-based hydrogels and nanoparticles in the treatment of *S. mutans* biofilms. One particular article took advantage of chitosan's ability to entrap peptides with stability issues [79]. They investigated the amelogenin-derived peptide QP5, which has been previously shown to promote remineralization. However, it was not effective at reducing the bacterial growth of *S. mutans* because of its short residence time [79]. Hydrogels were prepared with 1% medium molecular weight chitosan, 0.1 M $CaCl_2$, and 0.1 M Na_2HPO_4, and the pH was adjusted to 6.0. Bovine enamel blocks were treated for 5 min with one of the following formulations: QP5-loaded chitosan hydrogel, 25 μM QP5, 1000 ppm sodium fluoride (positive control), 1% chitosan (vehicle control), or water (negative control). The bacterial inoculum was added and incubated for 1, 4, or 7 days. The ability of the treatment to exert antibacterial activity and inhibit lactic acid production was evaluated for 7 days.

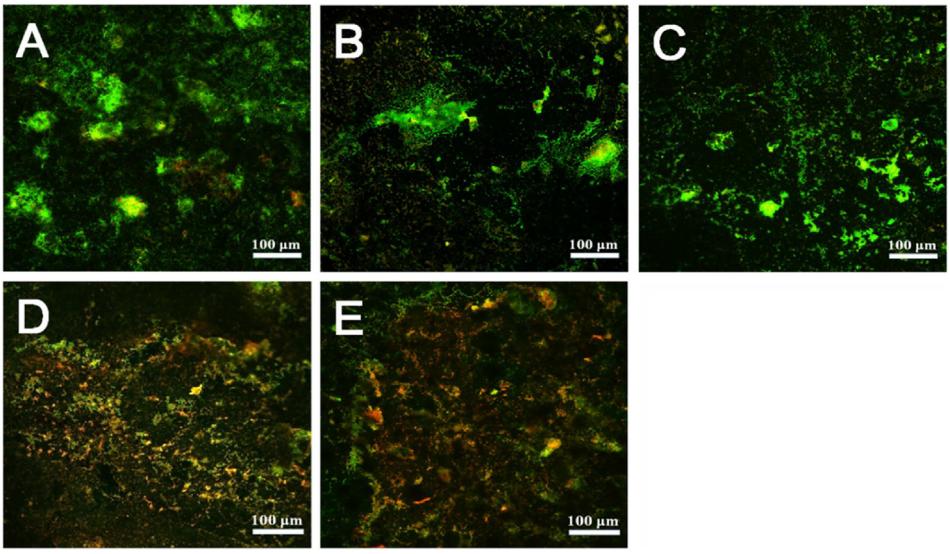

Fig. 4. "The impact of various dendrimers on the biofilm of *Streptococcus mutans* for 48 h was analyzed by laser confocal microscopy (green represents live bacteria, red represents dead bacteria). The substrates were treated by: A.) PBS buffer, B.) 3rd generation dendrimers (control), C.) 4th generation dendrimers (control), D.) apigenin loaded 3rd generation dendrimers (treatment), E.) apigenin loaded 4th generation dendrimers (treatment). Reproduced with permission from Ref. [33]. (For interpretation of the references to color in this figure legend, the reader is referred to the Web version of this article.)

In terms of antibacterial activity, the QP5-loaded chitosan hydrogel substantially inhibited the growth of the biofilm, as demonstrated by the significant reduction in colony-forming units. Sodium fluoride (control) can reduce the number of colony-forming units, although bacterial counts were higher than that of the QP5-loaded chitosan hydrogel. The non-formulated QP5 showed almost no antibacterial activity. With regard to lactic acid production, both 1% chitosan and the QP5 loaded chitosan hydrogel continuously and significantly decreased acid production over the 7-day treatment period [79]. Although similar effects were observed with QP5, it could not inhibit the production of acid to the extent of the QP5-loaded chitosan hydrogel. Sodium fluoride decreased lactic acid production on the first day; however, increasing the treatment duration did not result in an additional decrease in lactic acid production. Significantly, after a 7-day treatment period, the QP5-loaded chitosan hydrogel demonstrated the highest microhardness values compared to the other formulations, including the blank chitosan hydrogels.

In the formation of dental caries, acid-producing and acid-tolerant bacteria accumulate [80]. As the pH becomes more acidic over time, it allows chitosan to provide electrostatic interactions with QP5, preventing the early release of QP5 [79]. During this time, chitosan itself likely acts as an antibacterial agent by interacting with the negatively charged bacterial cell wall, causing bacterial cell death. As the pH shifts to a more neutral pH, QP5 is released, facilitating remineralization. This study showed that the QP5-chitosan hydrogel was more effective than the vehicle (1% chitosan).

5. Conclusions

Nanotechnology-based formulation approaches have been shown to improve therapeutic outcomes across different disease states. This review emphasizes the advances in the prevention, management, and treatment of biofilm-associated dental caries. The highlighted formulation types demonstrated improved therapeutic outcomes by enhancing drug solubility, promoting penetration into the deep layers of the biofilm, facilitating prolonged residence time in the buccal cavity, and reducing the emergence of drug-resistant phenotypes. In summary, these formulations have many potentials and provide new life to therapeutic agents with limited physicochemical characteristics.

Ethical statement

This article has not been published in whole or elsewhere. All authors contributed equally to this work.

CRediT authorship contribution statement

Felix Amissah: Conceptualization, literature search, Writing – original draft, Writing – review & editing. **Terrick Andey:** Conceptualization, literature search, Writing – original draft, Writing – review & editing. **Kristen M. Ahlschwede:** Conceptualization, literature search, Writing – original draft, Writing – review & editing, tables, two figures.

Conflict of interest

The authors declare that there are no conflicts of interest.

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