Chapter 9

Biomaterials in Caries Prevention and Treatment

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Abstract Dental caries is one of the most widespread diseases in humans and has become a heavy economic burden. Tremendous progress aiming at improving the clinical performance of dental composite has been made in the past decades but secondary caries still remains a main problematic issue in composite restoration failure. Recently, some novel biomaterials have been developed in caries prevention and treatment and showed broad prospects of application. Nanoparticles of amorphous calcium phosphate (NACP) and CaF_2 were synthesized and could release calcium/phosphate and fluoride ions, contributing to remineralize tooth lesions and neutralize acids. Biomineralization agents, PAMAM, could mimic the natural mineralization process of dental hard tissues. Quaternary ammonium methacrylates (QAMs) were identified to be effective against dental biofilms. These biomaterials are promising for incorporation into dental composite in preventive and restorative dentistry, thus improving the efficacy and success rate in caries prevention and treatment.

Keywords Dental caries • Dental materials • Mineralization • Antibacterial • Quaternary ammonium methacrylates • Calcium phosphate nanoparticles

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Dental caries is a dietary carbohydrate-modified bacterial infectious disease caused by biofilm acids and is one of the most prevalent chronic diseases of people worldwide [1–4]. It's the main cause of oral pain and tooth loss, affecting the oral health of human beings seriously and also creating a heavy financial burden; meanwhile, it has been associated with some systematic diseases as well [5, 6]. Basically, dental caries is a multifactorial disease, but demineralization of susceptible dental hard tissues resulting from acidic by-products from bacterial fermentation of dietary carbohydrates is considered to be the fundamental mechanism. Once biofilm acids have caused tooth decay, the most effective treatment currently is to remove the carious tissues and fill the tooth cavity with a restorative material [7, 8].

Resin-based dental composites are increasingly used for tooth cavity restoration due to their excellent esthetics and improved performance [9–12]. Significant progress has been made to equip the esthetic composite restorations with less removal of tooth structures, enhanced load-bearing properties, and improved clinical performance [13–16]. However, as composites tend to accumulate more biofilms/plaques in vivo than other restorative materials, plaque adjacent to the restoration margins could lead to secondary caries [17, 18]. Indeed, previous studies demonstrated that secondary caries and bulk fracture are considered to be the most important factors leading to dental composite restoration failure, among which secondary caries are a main reason for replacing the existing restoration materials [19, 20]. As a result, half of all dental restorations fail within 10 years, and nearly 60% of the average dentist's practice time is expended on replacing them [21, 22]. Replacement dentistry costs \$5 billion annually in the USA alone [23]. Therefore, there is a great need to explore novel anticaries materials to combat secondary caries by incorporating bioactive agents possessing remineralization and antibacterial properties into the resin composites and bonding systems.

9.1 Synthesis of Novel Mineralization Materials in Preventive Dentistry

In the normal status, there is a physiological equilibrium between the remineralization and demineralization of dental hard tissues in the oral cavity [24–26]. The equilibrium will break toward demineralization when the organic acids produced by bacteria increase to a certain extent [7]. In consideration of this, it would be beneficial to employ mineralization materials in caries prevention. To date, two main strategies have been carried out to remineralize demineralized dental hard tissues. One is the ion-based strategy, and the other is the use of biomineralization agents.

Composites containing calcium phosphate (CaP) composites were previously synthesized [27, 28]; these composites could release supersaturating levels of calcium (Ca) and phosphate (PO_4) ions and achieve remineralization of tooth lesions in vitro. More recently, nanoparticles of amorphous calcium phosphate (NACP) of about 100 nm in size were firstly synthesized via a spray-drying technique [29].

Owing to the small size and high surface area effects, the “smart” NACP nanoparticles exhibit excellent characteristics, dramatically increasing the calcium and phosphate ions released at a cariogenic low pH while possessing mechanical properties nearly twofold of abovementioned CaP composite. In addition, NACP nanoparticles could neutralize a lactic acid solution of pH 4 by increasing the pH rapidly to nearly 6, which could avoid caries formation [30, 31]. Besides NACP, nanocomposites containing CaF_2 nanoparticles were also developed [32]. Studies have shown that these novel nanocomposites can release calcium and fluoride ions in a long-term stable manner. Both calcium and fluoride ions can inhibit demineralization and promote remineralization of dental hard tissues, and fluoride also has some antibacterial activity by interfering the formation and metabolism of dental plaque biofilm [33–35].

Although employed widely in the remineralization of carious dentin, such an ion-based strategy cannot be effective in locations where the crystallites are totally destroyed [36]. Another promising class of mineralization materials is the biomineralization agents. Inspired from the function of non-collagenous proteins (NCPs) in the biomineralization process of natural teeth, using biomimetic templates to remineralize the demineralized dentin is of great interest in recent years as NCPs, the natural nucleation templates, lose their abilities to induce *in situ* remineralization in the mature dentin [37, 38]. Poly(amino amine) (PAMAM)-type dendrimer is widely studied in dental biomineralization. It is a class of monodispersed polymeric nanomaterials with plenty of branches radiating from one central core and highly ordered architecture. It has been referred as “artificial protein” due to its biomimetic properties and well-defined/easily tailored structure, such as its functional group, generation, and spatial structure. Previous studies [39, 40] have clearly demonstrated that PAMAM and its derivatives could induce biomineralization of the demineralized dentin. Combined PAMAM with antibacterial agents also obtained double effects of mineralization, and antibacterial and needlelike crystals can precipitate both on the dentin surface and in the dentinal tubules, suggesting that PAMAM is a promising biomineralization material for dental use.

9.2 Quaternary Ammonium Methacrylates (QAMs)

Quaternary ammonium salts (QAMs), widely used in water treatment, food industry, textiles, and surface coating because of their low toxicity and broad spectrum of antimicrobial activity, are some ionic compounds which can be regarded as derived from ammonium compounds by replacing the hydrogen atoms with alkyl groups of different chain lengths [41]. They were first introduced to dental material industry in the 1970s as mouth rinses [42, 43]. Since then numerous researchers have devoted to exploring novel QAMs to meet the “immobilized bactericide” [44, 45] concept in dental materials. To date, a great many kinds of QAMs, such as 12-methacryloylox ydodecylpyridinium bromide (MDPB) [44, 46, 47], methacryloxylethylcetylamm onium chloride (DMAE-CB) [48], quaternary ammonium dimethacrylate (QADM)

[49, 50], and quaternary ammonium polyethylenimine (QPEI) [51], have already been synthesized and incorporated into dental materials including glass ionomer cement (GIC) [52], etching-bonding systems [53, 54], and resin composites [55], attempting to achieve antibacterial effect. Recent researches have given us evidences that they make promising dental materials with properties which are suitable for treatments of dental caries and with much potential for preventing secondary caries.

9.2.1 Killing Bacteria and Inhibiting Biofilms

Many of the QAMs have been proven to be efficient in killing bacteria or inhibiting biofilms, including methacryloxyethylcetylammmonium chloride (DMAE-CB), quaternary ammonium dimethacrylate (QADM), quaternary ammonium polyethylenimine, and quaternary ammonium dimethacrylate. An *in vitro* [49] study which intended to develop novel antibacterial dentin primers containing both quaternary ammonium dimethacrylate (QADM) and nanoparticles of silver (NAg) proved that uncured primer could kill the planktonic bacteria in caries cavity, as well as inhibit dental biofilms.

Recently, it has been revealed that glass ionomer cements (GIC) containing different concentrations of a novel material – dimethylaminododecyl methacrylate (DMADDM) – which also belongs to the QAM family, have antibacterial effects to different extents as well [56]. The results of this study indicated that with DMADDM, both the live bacteria and the EPS decreased in the biofilms of *S. mutans*. And the group with the higher concentration had more significant effect. Consistent with the reduced live bacteria and EPS production, the glucosyltransferase-encoding genes, *gtfB*, *gtfC*, and *gtfD*, of *S. mutans* were also significantly downregulated after adding DMADDM. Another research [57] found that with DMADDM added to dental adhesives, the ratio of *S. mutans* steadily dropped in the multispecies biofilms of *Streptococcus mutans*, *Streptococcus gordonii*, and *Streptococcus sanguinis*.

9.2.2 Mechanical Properties

The mechanical properties are critical indexes for the evaluation antimicrobial modified dental materials, such as dental adhesives and resin composites. QAMs have been proved that they did not change the adhesion and strength of the dental materials. For example, the SEM examination of dentin-adhesive interfaces of a QADM modified dental adhesive revealed numerous resin tags “T” with no difference with the control [49]. Only at high mass fraction, QADM decreased the strength of the tetracalcium phosphate (TTCP) composite. Nevertheless, QADM can guarantee

both the antibacterial and the mechanical properties of the material at suitable concentrations [58].

9.2.3 Durability

Resin aging is another reason for secondary caries or failure of restorations. Accordingly, tests for durability are needed if QAMs are making a difference in this aspect of composites.

A recent study [50] compared the flexural strength and elastic modulus of a novel nanocomposite containing nanoparticles of amorphous calcium phosphate (NACP) and quaternary ammonium dimethacrylate (QADM) with two different commercial composites in water immersion. Like the other two composites, the new nanocomposite displayed a moderate decrease during the first month of aging, with little decrease during 1–6 months. After a 180-day immersion, the strength and modulus of NACP-QADM nanocomposite were similar to those of commercial control composites. Also, NACP-QADM nanocomposite maintained its antibacterial properties during immersion. These statistics indicated that composites with QAMs maintain the same level of durability as common commercial composites.

9.2.4 Biological Safety

Researchers recently have provided us with the human gingival fibroblast cytotoxicity data of a certain kind of QAMs [59]. In this study, the control had fibroblast culture in fibroblast medium (FM) without resin eluents, and its cell viability was set at 100%. For groups with resins, the fibroblasts were cultured in FM containing resin eluents. The fibroblast viability for all the bonding agent groups was nearly 100%. There was generally little difference between 12-methacryloyloxydodecylpyridinium bromide (MDPB) and NAg groups and the non-antibacterial adhesive control or between bonding agent groups and the FM control without resin eluents ($p > 0.1$). Another measurement [52] of the DMADDm release showed that DMADDm concentrations decreased continuously and after 12 h values were found near to the zero level for both 1.1 wt.% and 2.2 wt.% DMADDm containing specimens in water. And in saliva, only one set presenting ten specimens of 2.2 wt.% DMADDm revealed release of the substance near the detection level.

With 12-methacryloyloxydodecylpyridinium bromide (MDPB) displaying no more fibroblast cytotoxicity than the routine medium and DMADDm's low release rate and time, we may draw the conclusion that QAMs are biologically safe with humans. But more researches are needed to further explain it.

9.2.5 *Inhibiting MMPs*

Proteolytic degradation of hybrid layer by host-derived matrix metalloproteinases (MMPs) leading to reduction of resin-dentin bond strength is believed to be among the major reasons for the failure of resin restorations [60, 61]. Fortunately, it has been found that the resin-dentin bonding systems containing DMADDM inhibited MMPs efficaciously.

Assay of the loss mass of demineralized dentin beams revealed that dentin incubated in DMADDM lost significantly less mass than the control group at 7 or 30 days. While with both DMADDM and chlorhexidine digluconate (CHX), dentin mass dropped even sharply. And spectrophotometrical measurement of hydroxyproline (HYP), used to determine the dissolution of collagen peptides, reflected that the combination of DMADDM and CHX dramatically lowered hydroxyproline dissolution weight [62].

9.2.6 *Mechanism*

The antibacterial mechanism of quaternary ammonium methacrylates (QAMs) is now widely thought to be “contact killing.” That is, the positively charged (N^+) sites of the QAMs molecules would attract the negatively charged bacterial cells, and once contact is made, the electric balance of the cell membrane could be disturbed and the bacterium could explode under its own osmotic pressure. Previous studies [56, 63, 64] have confirmed that the surface charge density of the tested materials increased with the increase of the mass fraction of DMADDM, and higher mass fraction showed stronger antibacterial effects. A recent study [65] on the antibacterial effects of quaternary ammonium chain length revealed the increasing antibacterial potency with increasing CL from 3 to 16 while CL 18 may be a cutoff point. It was supposed that long-chained quaternary ammonium compounds could have additional antimicrobial activity by insertion into the bacterial membrane, resulting in physical disruption [66]. The evaluation of three-dimensional (3D) biofilms on antibacterial bonding agents containing QAMs showed that not only the bacteria contacting the resin surface but also through the bacteria in the 3D biofilm away from the resin surface were killed [67]. It was suggested that a stress condition in the bacteria could trigger a built-in suicide program in the biofilm, which was also termed the programmed cell death. However, the exact mechanism of QAMs still remained unclear and needed further research in the future.

9.2.7 *Resistant/Persister Bacteria*

Although the strong killing effects of QAMs are clinically beneficial, their frequent use may also have the possibility of leading to the development of bacterial drug resistance and tolerance as with other antibiotics, which are associated with the failure of antibacterial treatment and the relapse of many chronic infections. No reports have reported or investigated the possibility that oral bacteria can acquire resistance to QAMs so far. However, a new study recently has found that *Streptococcus mutans* could form a small portion of persisters which can survive lethal doses of DMADD treatment. Persisters are nongrowing dormant cells that are produced in a clonal population of genetically identical cells and are generally believed to be responsible for drug tolerance [68, 69]. In contrast to the drug-resistant bacteria, persisters are not mutants but phenotypic variants of the wild-type population. The isolated persisters in the abovementioned study have the typical characteristic of multiple drug tolerance, and the experimental drugs of different mechanisms are proven to have little effects on the persisters. This finding will partially challenge the clinical use of QAMs, and further studies are needed to elucidate possible mechanisms behind this in order to tackle the problem.

9.2.8 *Models*

Different models were used to study the biomaterials in caries preventions and treatment. Most of the investigations applied in vitro models. Besides, an individual removable acrylic upper jaw splint in situ has already been built to test some properties of QAMs including antibacterial effect and biological safety [52]. Transparent custom-made acrylic splints (Thermoforming foils®, Erkodent, Pfalzgrafenweiler, Germany) were fabricated from alginate impressions as carrier of the GIC specimens. Six samples were fixed in the left and right buccal position in the molar and premolar regions with silicon impression material (President Light Body®, Coltène, Altstätten, Switzerland) onto the splints for intraoral exposure. Animal models are under construction and more figures are coming out soon.

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