Mini Review: Novel antimicrobial compounds in the age of increasing bacterial resistance

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In recent years, increasing numbers of serious infections caused by antibiotic resistant bacteria have necessitated the development of new and effective antimicrobials. Antimicrobial peptides (human defensins and cathelicidin), ammonium polyethylenimine, zinc oxide and metal titanates have emerged as promising alternatives to conventional antibiotic therapies. These new classes of antimicrobials have novel mechanisms of action that ensure microbes have little chance to develop resistance. This review will focus on the antimicrobial properties of these compounds and examine their therapeutic potential.

Keywords: Antimicrobial peptides; titanate; infection treatment; bio-delivery; metal-based drugs; antibiotics

1. Introduction

The human body, in particular the skin and the oral cavity, is constantly exposed to a variety of bacteria, but most individuals maintain a healthy homeostasis. As one of the first host tissues to encounter colonizing bacteria, epithelia respond to the presence of bacteria through an elaborate signaling network, producing antimicrobial peptides (AMPs) and cytokines that trigger host innate immune responses. As bacterial resistance to systemic antibiotics has emerged as an increasing problem in the treatment of infections in recent years, development of suitable alternatives to replace or at least to support systemic antibiotic therapy is much needed. AMPs, known to be synthesized in all human epithelia tested to date, have shown a broad spectrum of activity against bacteria, yeast and viruses. Because these AMPs exhibit low risk for developing bacterial resistance, they are promising alternatives to the current antibiotic therapies. In addition, novel materials to treat recurrent oral infections have been developed in recent years. They include ammonium polyethylenimine, zinc oxide and metal titanates. These materials exhibit antibacterial activity against various oral pathogens, including those that cause periodontitis, caries and endodontic infections, suggesting they can be developed as a novel class of antibacterials. In this review, we will closely examine the effectiveness of AMPs against various diseases of skin and oral mucosa and also will focus on the future therapeutic potentials of these AMPs as well as other novel antibacterial compounds such as metal titanates.

2. Antimicrobial Properties of Antimicrobial Peptides (AMPs)

AMPs are small proteins (<100 amino acids) with a broad spectrum of activity against both Gram-negative and Gram-positive bacteria as well as against yeast and viruses [1, 2]. AMPs in humans include a cathelicidin family member LL-37 and defensins, such as α-defensins of intestinal and neutrophil origin, and the human-β-defensins (hBDs) of skin and oral mucosa and other epithelia [2-4]. The defensins are secreted in biological fluids, including urine, bronchial fluids, nasal secretions, saliva and gingival crevicular fluid, and in particular, hBDs are found in many epithelia including kidney and urinary tract, oral mucosa and skin [5-10]. Unlike hBD-1, whose expression is constitutive, the expression of hBD-2 and hBD-3 is inducible when epithelia are exposed to bacteria, Candida albicans, IL-1, or TNF-α [11-14]. In most tissues, hBD-2 is induced and expressed only in inflamed sites, whereas in the oral epithelium it also is expressed in normal uninfamed gingival tissue, presumably due to the constant exposure of this tissue to non-pathogenic commensal bacteria [15]. The mode of antimicrobial activity of AMPs has been most commonly attributed to disruption of cell membranes [16, 17], but a recent study has found that defensins can inhibit cell wall biosynthesis via binding and sequestering of lipid II, a building block of bacterial cell wall [18].

AMPs are known to play an important role in maintaining epithelial innate immunity, and especially in the oral cavity where multitudes of bacterial species are present. These peptides help maintain the balance between commensal and pathogenic species. Increased hBD-1 protein expression, as a result of the presence of a SNP on the hBD-1 gene, has been thought to provide protection against oral Candida, suggesting a significant role for AMPs in individuals susceptible to opportunistic infections [19, 20]. Antimicrobial activity of AMPs against other oral microbes has also been shown in various in vitro studies. For example, various Streptococcus species implicated in caries are susceptible to hBD-2, hBD-3 and LL-37 [21-24], while periodontal pathogens Porphyromonas gingivalis and Aggregatibacter
**3. Zinc Oxide and Polyethyleneimine Nanoparticles as Antimicrobials**

Zinc Oxide particulate (ZnO) has long been known as an antibacterial [46], but a recent development in reducing the particle size to nanoscale has produced even more effective antibacterial properties. ZnO nanoparticles (ZnO-NPs) have activities against various bacteria, including *Proteus vulgaris*, *Salmonella typhi*um, *Shigella flexineri*, *Bacillus cereus*, *Klebsiella pneumoniae*, *Campylobacter jejuni*, *Escherichia coli* and MRSA, suggesting these particles can be developed into effective antimicrobial agents against a broad range of infections [47-50]. The mechanism of antimicrobial activity of ZnO-NPs is thought to be via generation of reactive oxygen species, such as hydrogen peroxide (H\(_2\)O\(_2\)), much like the antimicrobial properties found among titanium dioxide nanoparticles [51-53]. In addition, ZnO-NPs incorporated into polymer biomaterials significantly reduced the growth of *S. aureus*, suggesting incorporation of ZnO-NPs in biomaterials commonly used for endotracheal tubes and implanted biomaterials may reduce common bacterial infection [54]. This is a noteworthy improvement in a quest for new and effective antimicrobials, as antibiotic resistant bacterial infection poses as one of the most common reasons for orthopedic implant failure [54, 55].

Composite resins are commonly used in restoration of tooth structure, but the shrinkage during polymerization often leads to micro-gaps between the resin and the tooth, either initially or after some period of intraoral service [56, 57]. Bacterial biofilm accumulation in these marginal gaps is a major cause of secondary caries, thus incorporation of antimicrobials into resin composite restorations would be advantageous in preventing recurrent caries, restoration failure, and loss of significant tooth structure [58, 59]. Addition of ZnO-NPs to composite resins has advantage over other commonly incorporated antimicrobial materials, such as chlorhexidine, fluoride or silver. Incorporation of ZnO-NPs resulted not only in enhanced antibacterial activity, but also in longer service life and lower degradation of resin composite restorations [57, 59].

Quaternary ammonium polyethyleneimine (QPEI) kills bacteria by disrupting their cell membranes, and many studies have demonstrated the antimicrobial efficacy of QPEI [58, 60, 61]. Incorporation of QPEI nanoparticles into dental resin composites at 1 wt% concentration has been effective against *Streptococcus mutans*, a major causative agent of dental caries, as well as against biofilm formation in vivo [58, 62]. QPEI at 2 wt% concentration also showed long-lasting antimicrobial effects against clinical isolates of *S. aureus*, *Staphylococcus epidermidis*, *Enterococcus faecalis*, *Pseudomonas aeruginosa* and *E. coli* with little effect on composite biocompatibility [63]. An interesting note...
on the antimicrobial effect of QPEI is that more than 50% of the bacteria in biofilms formed on QPEI-containing resin composites were dead and that this antimicrobial effect was not limited to the bacteria which came in direct contact with the material [58]. Because the incorporated QPEI nanoparticles are stable and not known to leach into the surrounding tissues, it is plausible that the effect of QPEI reaches beyond the immediate surroundings and into the other layers of the biofilm, possibly via an additional mechanism other than cell membrane disruption, suggesting a wide-reaching role QPEI may play as an anti-biofilm agent [58].

4. Metal Titanates as Novel Antimicrobial Compounds

Two forms of titanates, amorphous peroxotitanate (APT) and monosodium titanate (MST), are inorganic ion exchangers that can readily exchange a variety of metal ions for sodium to produce the corresponding metal titanate [64, 65]. MST is prepared using a sol-gel synthetic method that allows continued particle growth by simultaneous and controlled rate additions of a solution containing titanium(IV) tetraisopropoxide and sodium methoxide [66]. The resulting MST solids are spherically-shaped particles that range in size from about 1 to 20 microns with inner amorphous core surrounding a nano-size fibrous fringe. APT is prepared by the addition of hydrogen peroxide to a suspension of MST [67, 68]. The APT solids have a pH value of 4.0 ± 0.1 and the final solids concentration of 15.0 ± 0.5 wt %. Metal exchanged titanates are prepared by contacting solutions of the metal salts with MST and APT solids, and the amount of metal loaded onto the titanates is controlled by quantitatively measuring the difference in metal concentrations of the metal loading solutions before and after contact with the titanate solids as the number of g of metal per g of MST or APT [64, 65, 69]. In the sodium form, APT and MST do not suppress cellular metabolism and exhibit minimal cytotoxicity in *in vitro* studies using fibroblasts and monocytes [64]. Furthermore, sodium titanates, *per se*, do not trigger cellular inflammatory responses, thus are well suited for delivering metal ions with little alteration of cell function [64].

As recurrent caries at tooth-restoration interfaces are a major cause of restoration failure, it would be advantageous to incorporate effective antimicrobials in dental materials such as sealants, composites and bonding agents. Many metal ions inhibit the growth of bacteria, but systemic toxicity resulting from exposure to metal ions is an intolerable side-effect that has limited the therapeutic use of metals. However, metal titanate compounds inhibit the growth of various oral bacteria, and have emerged as a potential new class of antimicrobials. Titanate particles bind a variety of metals with high affinity and are not soluble in physiological settings. Thus, these materials are capable of sequestering and delivering metal compounds with less systemic toxicity [69]. Previous studies have demonstrated that metal titanates are delivered to fibroblasts as well as to monocytes [65, 69]. Although the sodium titanate particles alone do not suppress bacterial growth, titanates containing gold [Au(III)], platinum [Pt(IV)] or palladium [Pd(II)] ions inhibit the growth of a broad range of bacteria [70]. These compounds are inhibitory against; periodontal pathogens *P. gingivalis*, *A. actinomyctetomcomitans* and *Prevotella intermedia*; cariogenic bacteria *S. mutans* and *Actinomyces naeslundii*; *F. nucleatum*, a bridging organism between pathogens and non-pathogens; and non-pathogen *Streptococcus gordonii* [70]. The degree of inhibition varied among metals and bacterial species, but the inhibitory effect was enhanced 5- to 375-fold when metals were bound to titanates compared to metal ions alone, suggesting titanates augment metal-bacteria interactions [70]. Because the metal complexes are inorganic, little degradation is expected when incorporated into dental resin composites and therefore, they may have long-term effectiveness at a tooth-restoration interface. Potential bacterial resistance is also unlikely, because of the unique binding, coordination, and redox properties of metals, making titanate-metal complexes promising novel antimicrobial agents.

5. Potential Therapeutic Values of New Antimicrobial Materials

The mode of antimicrobial activity of conventional antibiotics is to disrupt cellular functions of bacteria without disrupting their cell walls. This mode of action keeps the bacterial morphology intact, ultimately leading to development of antibiotic resistance among many species. On the other hand, cationic AMPs bind to microbial surfaces and disrupt bacterial cell membranes via electrostatic interaction [71], and little bacterial resistance to AMPs has been reported thus far. AMPs not only show a broad spectrum of antimicrobial activities, but also are synthesized in various body sites, making them promising alternatives to the current therapy [72, 73]. Other beneficial properties of AMPs include utilization as adjuvants to enhance antibody production and as a link between the innate and the acquired immune systems. AMPs are chemotactic, thus can stimulate the acquired immune system and induce the release of pro-inflammatory mediators from epithelial and other cells [74-77]. The effectiveness of human AMPs has been tested in several animal studies. A subcutaneous administration of HNP-1 significantly reduced *Mycobacterium tuberculosis* infection in mice [78], whereas rats receiving a recombinant adenovirus carrying rat beta-defensin-2 were protected against sepsis or pneumonia induced by *P. aeruginosa* [79]. Although the effectiveness of AMPs in treating infections in humans has yet to be tested, numerous *in vitro* as well as animal studies strongly suggest human AMPs are a suitable platform for developing new therapeutic agents.

Recent years have seen increased development of nanoparticle designs as treatments for various diseases and infections. Although successful development of nanoparticles still faces numerous challenges including fine-tuning
their shapes and sizes as well as directing the particles to the right organs, the promise they hold is certainly noteworthy. As presented in this review, nanoparticles of ZnO and QPEI have proven effective antimicrobial activities against wide ranging microbes. In addition, inhibitory effects exhibited by micron-sized metal titanate particles against various types of bacteria suggest nanoparticles of these compounds will be even more potent, since they will have significantly higher surface-to-volume ratio for more effective ion-exchange. A potential application for the metal titanates would be to load composites or bonding agents with these particles and limit recurrent infection. These compounds might also be used to reduce bacterial counts in the periodontal pocket adjacent to materials containing metal-loaded titanates, as well as to prevent post-endodontic treatment periapical infections using titanate-loaded sealers and fillers.

In summary, recent studies present many roles new classes of antimicrobial materials play in disease and infection control. The effectiveness of these materials may be enhanced by the emerging ability to customize the particle size and mode of delivery. Future studies will further characterize the mechanism of bacterial growth inhibition and establish methods from several types of bio-material platforms to inhibit more complex biofilms.

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References


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