Nanostructures and Combating Oral Bacterial Diseases

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The oral cavity is an ideal environment for microbial cell growth, survival, and stability, followed by oral biofilm formation on the tooth surface. Biofilms contain a set of bacteria that are produced in extracellular polymeric substances (EPS). Bacterial growth results in the conversion of bacterial biofilm from commensal plaque to a pathogenic form. Bacteria present in the biofilm are significantly less sensitive to antimicrobial agents than planktonic bacteri.

Keywords: nanoparticles ; zinc oxide ; biofilms ; dental caries ; root canal therapy ; periodontitis ; antimicrobial photodynamic therapy ; drug delivery systems

1. Introduction

Biofilms have an important impact on humans in many ways as they can develop in natural, medical, and industrial environments [1]. Biofilm-associated cells differ in physiology and trigger different gene expression profiles to adapt to the biofilm environment [2]. For example, the genes SMU.629 and SMU.1591 encode superoxide dismutase and catabolite control protein A, which are involved in the oxidative stress tolerance and the expression of biofilm-associated genes in *Streptococcus mutans*, respectively. SMU.629 and SMU.1591 were impressively increased in biofilm [3][4].

The bacterial composition of dental biofilms is relatively stable and mainly consists of sites that are protected from biofilm-removing forces applied in the mouth [5]. Biofilms formed on the teeth produce diseases such as caries, chronic gingivitis, and periodontitis that have a wide range of side effects. If dental biofilms persist, they become harder and form calculus in the lower and upper gum areas, which cause an inflammatory response of the host and increase the amounts of tissue fluid flowing into gingival pockets and can lead to gum disease [6].

The inhibition of oral bacterial biofilms is challenging. The low pH milieu indicative of oral biofilms reduces the efficacy of antibiotics on oral biofilms. The acidic environment induces further EPS synthesis. The presence of EPS reduces drug access and triggers bacterial tolerance to antibiotics [7]. The mechanical removal of bacterial biofilms from dental surfaces is a proven method of treating periodontal diseases. However, mechanical procedures alone are not able to entirely kill all bacteria. Furthermore, the use of metal instruments to remove oral biofilms will result in tooth structure removal over time, and can ultimately lead to gum resorption and tooth sensitivity to physical and thermal stimulation-induced effects [8]. It has also been reported that chlorhexidine has many adverse effects ranging from mild symptoms such as headache and dry mouth to severe symptoms such as tooth staining, calculus formation, and upper respiratory tract infection and, therefore, is not suitable for daily, long-term use. Alternative anti-biofilm agents include alcoholic mouthwashes, which can irritate the oral mucosa and lead to oral cancer [9]. Additionally, natural therapeutic drugs have little efficacy due to poor drug solubility, and low penetration of the EPS matrix [7].

An alternative way for infection control has been the design and development of novel biocompatible and non-absorbable nanoparticles (NPs), which may reach high local bioactivity in a controlled release of antibacterial effects [10]. NPs have distinct advantages in that they exhibit special physical and chemical properties due to their ultra-small sizes and large surface area-to-mass ratio. This includes increased reactivity, greater solubility, biomimetic features and the ability to be functionalized with other substances such as drugs, bioactive molecules and photosensitizers. Furthermore, antimicrobial NPs can effectively infiltrate the oral biofilms, leading to the effective delivery of therapeutics and may help manage the use of antibiotics [11]. The large surface area and high charge density of NPs enable them to interact with negatively charged bacterial cells, causing enhanced antimicrobial activity, cell membrane damage, generation of reactive oxygen species (ROS), interference with cellular processes, proteins destruction, and, finally, cell death induced by DNA damage [12]. Despite several benefits, NPs have some disadvantages, such as toxicity, refraining from the physiological barrier, evading rapidly from the phagocytic cells, and mounting an immune response that should be carefully considered if they are used in living organisms [13].

Antimicrobial photodynamic therapy (aPDT), which combines a photosensitizer and a light source, has been suggested as an alternative technique for bacteria inactivation. To perform efficient aPDT, photosensitizers must be able to infiltrate bacteria [14]. NPs improve the effectiveness of the photosensitizer because they can pass through the bacteria cell walls [15]. Additionally, nanotechnology in the case of drug delivery vehicles can improve drug availability into the target tissue to deliver the maximum therapeutic effect [16]. Herein, this review investigated the antimicrobial actions of NPs against oral infectious disease with a focus on oral bacterial biofilms. Additionally, we will also elaborate on the role of NPs when applied in aPDT and drug delivery systems.

2. NPs and Their Role in Dental Caries Control

Dental caries is a multifactorial and biofilm-mediated oral disease [17]. The basic mechanism of dental caries is an ecological shift within the dental biofilm to acidogenic bacteria growth, frequently made by exposure to fermentable carbohydrates. The change in pH leads to an imbalance between de/remineralization processes, thus creating clinical decay [18]. *S. mutans* is the major bacterial species of dental caries [19], and it is capable of reducing environmental pH that results in the decalcification of the tooth [19]. Controlling dental caries can, therefore, be achieved through preventing the bacterial action, helping reverse tooth demineralization, and promoting the remineralization process [20]. The complete removal of carious dentin before placing a restoration often results in a pulpal exposure [21]. According to scientific evidence, every effort ideally should be made to maintain viable pulp tissue [22]. The use of nanostructures in dental caries management has also received much attention [23]. One study explored how chitosan (CS) combined with zinc oxide/zeolite (ZnO/Z) nanocomposite accelerates the biofilm reduction, metabolic activity and cariogenic properties of *S. mutans* [24]. The effect of ZnONPs on bacteria is through damage to the cell wall, lipids and protein, resulting in the leakage of intracellular contents to the outside of the bacteria and, ultimately, bacterial death [25].

Furthermore, recurrent caries is a major factor for restoration failure [26]. To address this problem, an adhesive containing NPs of amorphous calcium phosphate (NACP) was developed. NACP could enhance the Ca and P ions released under low pH conditions. Moreover, NACP adhesive could rapidly change the solution pH from 4.0 to above 5.5, improving the dentin remineralization. The NACP adhesive was also reported to show inhibition properties against bacteria and decrease the colony-forming units count in a biofilm of *S. mutans* [27].

Furthermore, metallic NPs are popular due to their antibacterial properties and biocidal activities at low concentrations. Copper NPs (CuNPs) and ZnONPs have been demonstrated to be effective against a wide range of bacteria. The addition of CuNP/ZnONP in an adhesive system provides more antimicrobial activities, without affecting their bond strength. Moreover, CuNP/ZnONP also inhibited matrix metalloprotease-2, and may improve enamel remineralization [28]. Matrix metalloprotease-2 is associated with the development of dental germ as well as the progression of dental caries in patients with necrotic pulp [29].

Another pertinent example is that of Cao and colleagues, who used a novel resin-based dental composite containing a photocurable core–shell of silver bromide (AgBr) plus cationic polymer nanocomposite (AgBr/BHPVP). The potent antibacterial effects of this resin composite against *S. mutans* are exerted through its biocide-releasing mechanism of the Ag^+ ions. Thus, resins containing AgBr/BHPVP NPs with long-term antimicrobial effects exhibited excellent potential as an antimicrobial agent which could be used in dental products [30].

3. NPs and Their Role in Root Canal Treatment

The failure of root canal treatment is attributed to the eradication of bacteria and incomplete disinfection of the complex root canal system, which will inevitably lead to persistent apical periodontitis. *Enterococcus faecalis* is a bacterium often isolated from the root-filled teeth with chronic apical periodontitis [31]. *E. faecalis* invades the dentinal tubules, adheres to the root canal wall and forms a biofilm on dentin [31]. Endodontic diseases are primarily associated with biofilm-mediated infection [32]. Microbial biofilms inside the root canal are very resistant to conventional medicaments including systemic antibiotics and sealers. The anatomical complexities and the multi-species biofilms increase the difficulty in the complete eradication of *E. faecalis* microbial biofilm. In recent years, the application of NPs to disinfect root canals has gained attention due to their broad spectrum antibacterial activity [33].

A study investigated the antibacterial activity of propolis NPs (300 µg/mL) with an average size of 117.6 nm as a root canal irrigant against a root canal infected with *E. faecalis* biofilm. Results confirmed propolis NPs were equally effective as NaOCl (6%) and chlorhexidine (2%) in reducing the *E. faecalis* biofilm [34]. Interestingly, an in vitro study exhibited promising results on root canal surfaces treated with cationic antibacterial NPs such as ZnONP, CS/ZnONP, or CS-layer-

ZnONP. But this effect was not considered efficient enough to elicit whether the inhibition of bacterial recolonization and biofilm formation was due to the killing of bacteria or by the direct effect of NPs on the bacteria–substrate interaction [35].

Recently, a novel antibacterial root canal sealer (dimethylaminohexadecyl methacrylate (DMAHDM)), and NACP were developed and tested on *E. faecalis* biofilm inhibition. There was a 3-log reduction in *E. faecalis* counts due to the new sealer compared to the control. Moreover, this root canal sealer demonstrated acid-neutralizing capabilities which could be useful in preventing anaerobic growth. The therapeutic root canal sealer, by releasing Ca and P ions, was able to inhibit *E. faecalis* biofilms. This structure, with antibacterial effects and remineralization capabilities, is a promising strategy to improve the success rate of endodontic therapy and dentin hardness [36].

4. NPs and Their Role in Periodontitis and Peri-Implantitis

Periodontitis, a chronic inflammatory condition, usually gradually destroys the tooth-supporting structures, subsequently leading to the looseness and, finally, the loss of teeth. Periodontitis is not only considered a major risk of further tooth loss, but is also reported to be associated with systemic disorders [37]. Periodontitis is one of the main risk factors that enhance the risk for peri-implantitis [38]. Peri-implantitis is an inflammatory disease affecting the tissues surrounding osseointegrated dental implants resulting in pocket formation, purulence and the loss of supporting bone, which is associated with the reduction of implant survival [39][40]. The accumulation of bacteria on the surface of implant and multi-species biofilm formation is considered the cause of dental implant failure and peri-implantitis [40].

Porphyromonas gingivalis, *Prevotella intermedia* and *Aggregatibacter actinomycetemcomitans* are the top three periodontitis and peri-implantitis-associated species in subgingival plaque. In the periodontal pockets, these bacteria can regulate the expression of many virulence factors that lead to supporting bone loss [41]. Recent studies have recently stated significant transcriptional changes when the above-mentioned bacteria were growing within a microbial multi-species biofilm [42][43][44]. Despite comprehensive mechanical and antimicrobial treatment, the long-term control of infection is impossible [45]. The use of NPs in the periodontal field is fascinating, particularly for infection control and oral biofilm management [46].

Carbon quantum dots are a new type of carbon-based nanomaterials which have unique properties such as high photostability, favorable water solubility, biocompatibility, low toxicity, and ease of preparation and modification. Liang and colleagues fabricated tinidazole carbon quantum dots using the hydrothermal method. The antimicrobial activity of tinidazole carbon quantum dots depends on its ability to impair toxicity by inhibiting the main virulence factors associated with the biofilm formation of *P. gingivalis* and, hence, affecting the self-assembly process of biofilm-related proteins [47].

Nanoscale surface modification has been suggested to affect bacterial adherence and biofilm formation on implants. Besinis et al. confirmed that a dual-layered silver plus nano-hydroxyapatite (HA) coating exhibited a strong inhibitory effect on the growth of *Streptococcus sanguinis* in the surrounding media and reduced the biofilm on the titanium-based implant surface by 97.5% [48]. *S. sanguinis* plays a significant role as the initial colonizer in the early adhesion stage of biofilm formation and titanium implant colonization, because it bonds directly to the implant surface and facilitates the later bacterial adhesion [49]. This novel nanocoating on titanium implants decreased infection risk, improved osseointegration, and enhanced bone healing [48].

5. NPs and Their Role in Orthodontics Infection Control

Fixed orthodontic treatments hamper the proper cleaning process, leading to biofilm buildup and white spot lesion formation. After orthodontic therapies with fixed appliances, there are significant increases in the amounts of cariogenic bacteria and biofilm formation [50]. Adding NPs to the orthodontic adhesives can reduce the frictional force between the orthodontic bracket and wire, improve the antimicrobial properties, and prevent enamel demineralization during orthodontic treatment [51].

The inhibitory effect of silver NPs in biofilm growth could predict that the silver NPs reduce biofilm metabolic activity [52]. Additionally, Xie et al. reported that the dental composite containing NACP considerably inhibited enamel demineralization and white spot lesions around orthodontic brackets [53]. Another study showed that orthodontic composites containing 1% titanium oxide NPs could significantly reduce the number of bacterial cells compared with conventional composites, without compromising the shear bond strength [54].

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