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REVIEW

ZnO and TiO_2 nanoparticles as novel antimicrobial agents for oral hygiene: a review

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Abstract Oral cavity is inhabited by more than 25,000 different bacterial phylotypes; some of them cause systemic infections in addition to dental and periodontal diseases. Emergence of multiple antibiotic resistance among these bacteria necessitates the development of alternative antimicrobial agents that are safe, stable, and relatively economic. This review focuses on the significance of metal oxide nanoparticles, especially zinc oxide and titanium dioxide nanoparticles as supplementary antimicrobials for controlling oral infections and biofilm formation. Indeed, the ZnO NPs and TiO_2 NPs have exhibited significant antimicrobial activity against oral bacteria at concentrations which is not toxic in in vivo toxicity assays. These nanoparticles are being produced at an industrial scale for use in a variety of commercial products including food products. Thus, the application of ZnO and TiO_2 NPs as nanoantibiotics for the development of mouthwashes, dental pastes, and other oral hygiene materials is envisaged. It is also suggested that these NPs could serve as healthier, innocuous, and effective alternative for controlling both the dental biofilms and oral planktonic bacteria with lesser side effects and antibiotic resistance.

Keywords ZnO and TiO_2 nanoparticles · Oral bacteria · Biofilm · Nanoantibiotics · Antibiotic resistance · Nanomedicine · Health effects

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Introduction

The oral microbiome is a complex ecosystem consisting of about 25,000 different bacterial phylotypes as revealed by deep sequencing of human oral microbiome, traditional cultivation, and cloning approaches (Jenkinson et al. 2011; Keijser et al. 2008; Liu et al. 2012; Paster et al. 2006). The proliferation of pathogenic bacteria within the mouth gives rise to periodontitis, an inflammatory disease, which also constitutes a risk factor for other systemic diseases (Zbinden et al. 2012) such as endocarditis and colorectal cancer (Han and Wang 2013). Therefore, one of the most urgent and important biomedical challenges of our times is to clarify the role of microbial communities in human health (Belda-Ferre et al. 2011; Turnbaugh et al. 2007; Ximénez-Fyvie et al. 2000). Unfortunately, the antibiotic therapies have rendered these bacteria resistant to traditional antibiotics (Leistevuo et al. 2000; Sweeney et al.

2004). Therefore, it has been envisaged that the metal oxide nanoparticles with antimicrobial activity (nanoantibiotics) could offer a good alternative to abate and/or control the growth of bacteria in oral cavity (Allaker 2010). This review discusses briefly the impact of oral cavity bacteria on oral and other systemic diseases and emphasizes the role and mechanism of metal oxide nanoparticles, particularly ZnO NPs and TiO_2 NPs in impeding the growth and biofilm formation activity of oral bacterial.

Oral bacteria: a health concern

Oral cavity is one of the most densely populated regions of human microbiome, where the heterogeneity of tissue types in the oral cavity, such as teeth, tongue, and mucosa, provides diverse ecological niches for the colonization of niche-specific microorganisms resulting in tongue coating, supragingival, and subgingival plaques (Fig. 1; Takahashi 2005; Kolenbrander and London 1993; Takahashi et al. 1997; Takahashi and Schachtele 1990). However, *Streptococcus mutans* and *Porphyromonas gingivalis* have received considerable attention due to their role in dental caries and periodontitis, respectively (Loesche 1986; Hayashi et al. 2010).

Dental caries, periodontal diseases, and microbes

Dental and periodontal diseases have multiple etiologies, but are largely caused by bacteria. Oral microbiome is one of the most extensively studied human microbiomes both for normal and diseased subjects (Nasidze et al. 2009; Wang et al. 2013a). Bacterial species belonging to 11 different phyla (*Actinobacteria*, *Bacteroidetes*, *Chloroflexi*, *Firmicutes*, *Fusobacteria*, *Proteobacteria*, *Spirochetes*, *Synergistetes*, *Tenericutes*, SR1, and TM7) inhabit the oral cavity (Aas et al. 2005; Dewhirst et al. 2010; Munson et al. 2004; Paster et al. 2001). High diversity of salivary microbiome within and between individuals has also been reported (Nasidze et al. 2009). Dental caries starts with the disturbance in the microbial homeostasis of the oral cavity and biofilm formation on the surface of the teeth. Bacteria initiate the biofilm formation by attaching firmly and irreversibly on dental surface and by evading the host defense system. Clarke identified *S. mutans* as one of the most important organisms causing dental caries, back in 1924. Lately, Socransky et al. (1998) have defined the red complex of bacteria (*Treponema denticola, P. gingivalis*, and *Tannerella forsythia*) associated with diseased site, and their collective ability to interfere with host defense mechanism. Among the primary

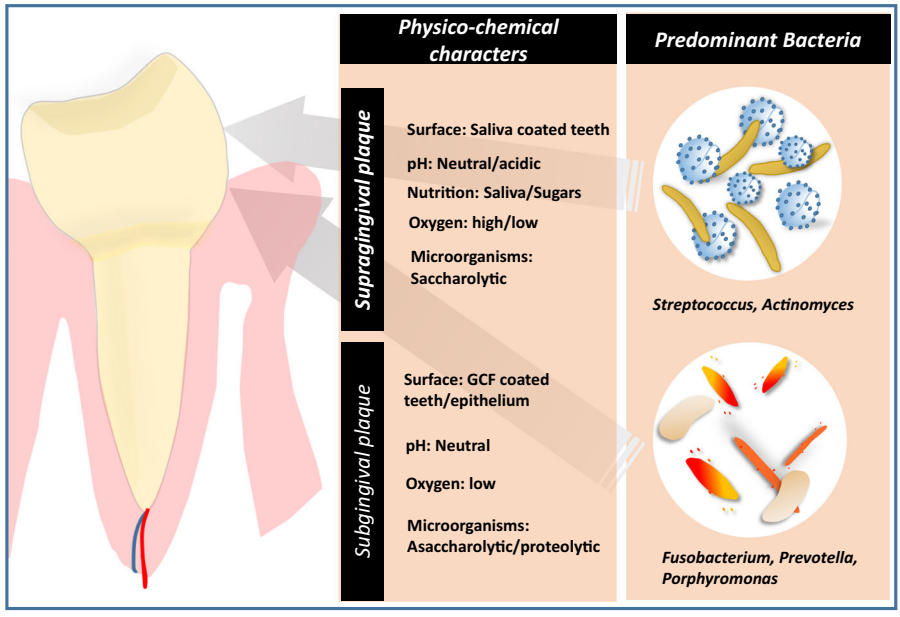

Fig. 1 Characteristics of ecological niches in the oral cavity supporting colonization of the niche-specific microorganisms

colonizers that initiate the biofilm formation are *S. oralis*, *S. mitis*, *S. sanguinis*, *S. parasanguinis*, *S. gordonii*, *Actinomyces*, *Veillonella*, *Gemella*, *Abiotrophia*, *Granulicatella,* and *Lactobacillus* (Darveau 2010; Hojo et al. 2009; Komoria et al. 2012; Kolenbrander et al. 2010; Socransky et al. 1998). These primary colonizers produce various biomolecules to overcome the host defense system such as leucotoxins (Höglund et al. 2014), proteases like immunoglobulin A1 protease, IL-8 protease (Cole et al. 1994; Frandsen et al. 1986; Kolenbrander et al. 2002; Zinkernagel et al. 2008), and glycosidases (Bradshaw et al. 1994) in addition to the substances required for their binding on oral surfaces such as exopolysaccharides, environmental DNA, proteins, and lipoproteins. Once the first layer of colonizers is established, secondary colonizers start binding on the surface of these primary colonizers. Binding of secondary colonizers quickly and effectively on a preformed *Streptococcus* biofilm has been demonstrated by Skopek et al. (1993). Among secondary colonizers are *P. gingivalis*, *P. intermedia, T. denticola*, *F. nucleatum*, *A. actinomycetemcomitans*, and *Lactobacillus* (Fig. 2). The biofilm utilizes dietary sugars and continuously produces acids causing demineralization of enamel and bone loss.

Oral bacteria and systemic diseases

Oral infections, specifically periodontitis, influence the progression and pathogenesis of many systemic diseases, including the cardiovascular disease, bacterial pneumonia, diabetes mellitus, and low birth weight (Ali et al. 2011; Bascones-Martinez et al. 2011; Teles and Wang 2011; Li et al. 2000). A detailed list of oral bacteria associated with various systemic infections is given in Table 1. Oral infections affect the host's susceptibility to systemic disease by causing transient bacteremia and metastatic injuries due to microbial toxins (Li et al. 2000). Lipopolysaccharides (LPS) from subgingival biofilms induce the vascular responses (Marcus and Hajjar 1993; Mattila 1989; Williams and Offenbacher 2000) and up-regulate the expression of endothelial cell adhesion molecules and secretion of interleukin-1 (IL-1), tumor necrosis factor alpha (TNF-α), and thromboxane. These mediators in circulation result in platelet aggregation and adhesion, formation of lipid-laden foam cells, deposits of cholesterol, preterm labor, and low birth weight infants (Herzberg and Meyer 1996; Han et al. 2010). *F. nucleatum* one of the predominant bacteria involved in adverse pregnancy outcomes has been isolated from fetal membranes, amniotic fluid, neonatal gastric aspirates, and fetal lung (Han et al. 2010; Han and Wang 2013). It has also been demonstrated that these *F. nucleatum* originated from mother's subgingival plaque (Han et al. 2010; Han and Wang 2013). *F. nucleatum* has also been associated with a number of other systemic diseases (Table 1), and its pathogenicity can be attributed to its ability to adhere to cell surface through FadA adhesin. FadA binds to VE-cadherin receptors on the surface of endothelial cells increasing their permeability thus allowing the penetration of bacteria into the cells (Fardini et al. 2011). Following penetration into the cell, *F. nucleatum* stimulates TLR4-mediated inflammatory response. *F. nucleatum* also promotes colorectal cancer by recruiting tumor-infiltrating immune cells and creating a microenvironment conducive for colorectal neoplasia progression (Kostic et al. 2013).

Another important bacterium involved in a number of systemic diseases is *P. gingivalis*. This bacterium can actively adhere to and invade endothelial cells including human umbilical vein endothelial cells (Deshpande et al. 1998). Its mechanism of pathogenicity and involvement in cardiovascular disease and atherosclerosis has been comprehensively reviewed (Hayashi et al. 2010). *P. gingivalis* often induces a local chronic inflammatory response by modulating complement system resulting in oral inflammation and bone destruction. Toll-like receptors play an important role in the initiation of this inflammatory response. One of the unique characters of *P. gingivalis* through which it manifests its pathogenicity is peptidylarginine deiminase.

Resistance to antimicrobial agents among oral bacteria

Resistance to some antibiotics commonly prescribed for oral infections (Beta-lactams, macrolides, tetracyclines, lincosamides, and nitroimidazoles) is widespread among oral bacteria even among healthy children (Dar-Odeh et al. 2010; Ready et al. 2003; Sweeney et al. 2004). Leistevuo et al. (2000) reported resistance to cefuroxime, penicillin, and tetracycline in 839 strains of *S. mutans*. In another study, β-Lactamase-producing strains including *Prevotella*

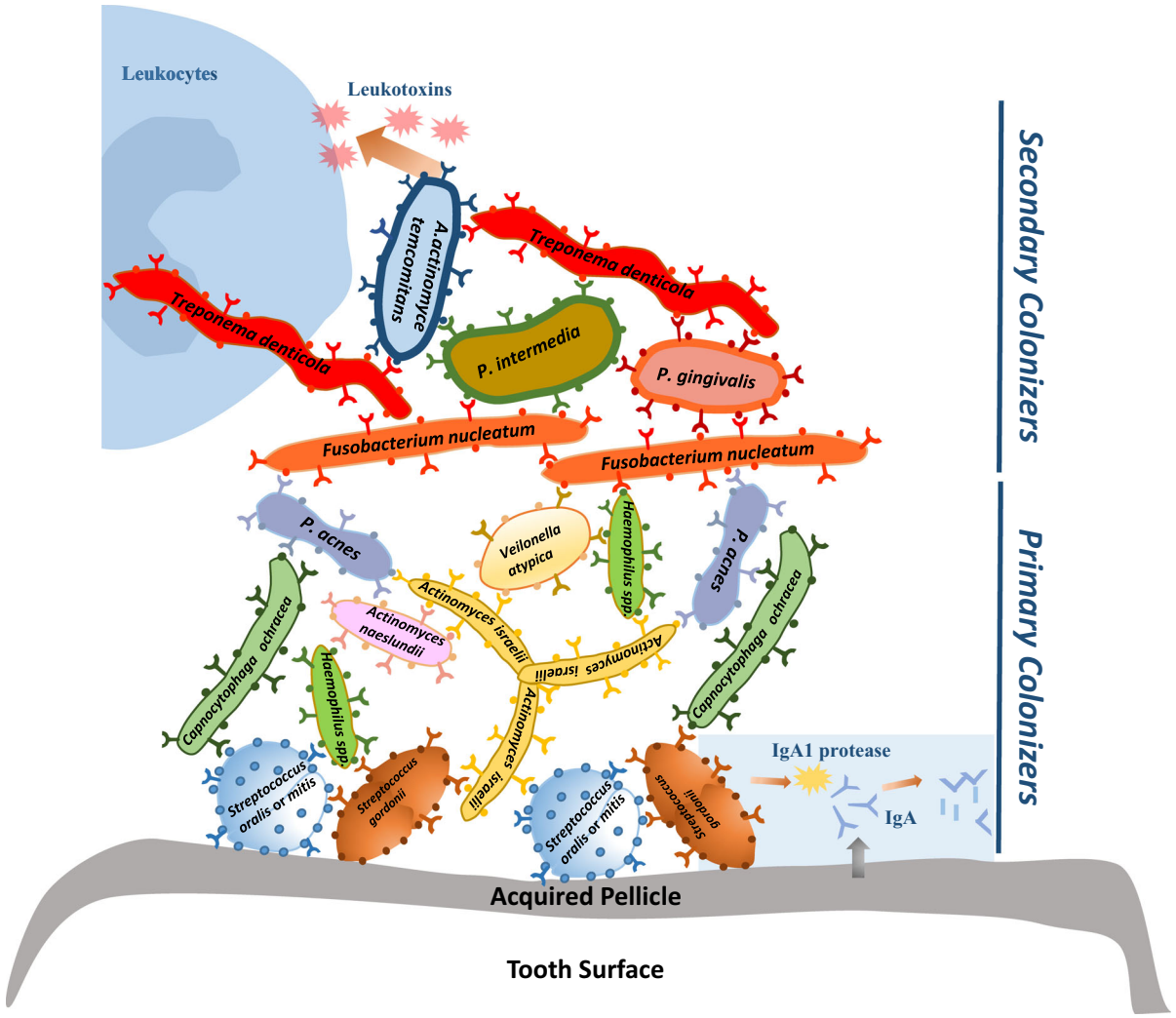

Fig. 2 Schematic presentation of biofilm formation by oral bacteria on the surface of the teeth

intermedia, P. denticola, and *F. nucleatum* were isolated from patients with dental caries (Fosse et al. 1999). Remarkably 80 % of the β-lactamase-producing *F. nucleatum* exhibited an MIC value as high as 8 mg/L (Nyfors et al. 2003). Greater resistance against the antibiotics clindamycin, metronidazole, and amoxicillin has been reported in *P. gingivalis* and *A. actinomycetemcomitans* associated with periodontal disease (Ardila et al. 2010). Significantly, higher incidence of resistance against spiramycin and metronidazole has been reported in periodontal *A. actinomycetemcomitans* strains (Madinier 1999). Even oral bacteria that are not directly involved in dental and/or periodontal diseases exhibit resistant to antimicrobial agents (Kouidhi et al. 2011; Villedieu et al. 2004). Rôças and Siqueira (2012) have reported a widespread distribution of antibiotic resistance genes in bacteria isolated from infected root canals. The most prevalent genes were β-lactamases bla_{TEM} (17 %), followed by tetracycline *tet*W (10 %) and macrolide erythromycin *erm*C (10 %). The prevalence of *tet*Q (tetracycline), *cep*A(β-lactamase), and *cbl*A(β-lactamase) genes has also been documented (Kirchner et al. 2013). Moreover, bacteria in oral biofilms are more resistant to antibiotics than in planktonic form (Mah and O'Toole 2001) due to the inability of antimicrobial agents to penetrate through the polymeric matrix secreted by the bacteria.

Table 1 Systemic diseases caused by oral bacteria

Disease	Causative agent	Reference
Cardiovascular disease	Circumstantial evidence	Herzberg and Meyer (1996), Dietrich et al. (2013)
Endocarditis	*Streptococcus tigurinus*	Zbinden et al. (2012)
Artherosclerosis	*F. nucleatum*, *Chlamydia pneumoniae, Veillonella, Streptococcus*	Lee et al. (2012), Byrne and Kalayoglul (1999), Koren et al. (2011)
Aspiration pneumonitis	*Actinomyces israelii*	Morris and Sewell (1994)
Adverse pregnancy	*F. nucleatum*	Han et al. (2010)
	Bergeyella sp.	Wang et al. (2013a, b)
Alzheimer's disease	Circumstantial evidence	Kamer et al. (2008)
Rheumatoid arthritis	*F. nucleatum, Serratia proteamaculans*	Témoin et al. (2012)
Oral and gastrointestinal carcinoma	*F. nucleatum, P. gingivalis*	Whitmore and Lamont (2014)
		Ahn et al. (2012)
		Castellarin et al. (2012)
		Kostic et al. (2013)
Inflammatory Bowel diseases	*F. nucleatum, C. concisus*	Ismail et al. (2012)

Nanoantibiotics: an alternative approach to combat antibiotic resistance

Pen (project on emerging nanotechnologies) project lists about 1824 commercial products including biomedicine that use nanomaterials (Berube et al. 2010). Plethora of biomedical applications has also been proposed for nanoparticles based on their unique physico-chemical properties. Their roles in hyperthermia treatment of cancer (Banobre-López et al. 2013), surgery (Ou et al. 2014), therapeutics (Zhang et al. 2008), biosensors (Luo et al. 2006), imaging (Drummen 2010), as drug carriers (De Jong and Borm 2008), and as anticancer (Cai et al. 2008) and antimicrobial agents (Marambio-Jones and Hoek 2010) are extensively reviewed. Nano-based drugs such as Emend, Rapamune, and Estrasorb are already approved by USFDA (Zhang et al. 2008) and are being marketed. One such nano-based product is nanoantibiotics. Nanomaterials that either exhibit antimicrobial activity per se or augment the efficacy and safe delivery of the antibiotics are called "nanoantibiotics" (Abeylath and Turos 2008; Huh and Kwon 2011; Kim et al. 2007). Nanoantibiotics offer significant benefits and advances in addressing the problems in treating infectious disease and hence are emerging as promising alternative antimicrobial agents. Some nanoparticles could be cost-effective (Li et al. 2008; Seyedmahmoudi et al. 2015), and are stable for long-term storage with a prolonged shelf-life (Weir et al. 2008). In addition, some NPs can withstand harsh conditions, such as high temperature sterilization, in comparison to conventional antibiotics (Applerot et al. 2012).

Mechanisms by which NPs exhibit their antimicrobial activity against bacteria include (i) disruption of bacterial cell membrane integrity (Xi and Bothun 2014), (ii) induction of oxidative stress by free radical formation (von Moos and Slaveykova 2014), (iii) mutagenesis (Ahmad et al. 2012), (iv) protein and DNA damage (Li et al. 2013), (v) inhibition of DNA replication by binding to DNA (Li et al. 2013), and (vi) respiratory chain disruption (Choi et al. 2008). Figure 3 depicts the plausible mechanisms by which metal or metal oxide NPs exhibit toxicity against bacteria and bacterial biofilm. The antimicrobial property of NPs largely arises and depends on their shape (Pal et al. 2007), size (Azam et al. 2012; Raghupathi et al. 2011), and the ability to form free biocidal metal ions (Song et al. 2010; Wang et al. 2010). Conversely, the sensitivity of bacteria to these NPs depends on their biochemical nature and composition, such as cell wall composition and growth rates. Baek and An (2011) reported that Gram-negative bacteria *Escherichia coli* are highly susceptible, whereas Gram-positive *Staphylococcus aureus* and

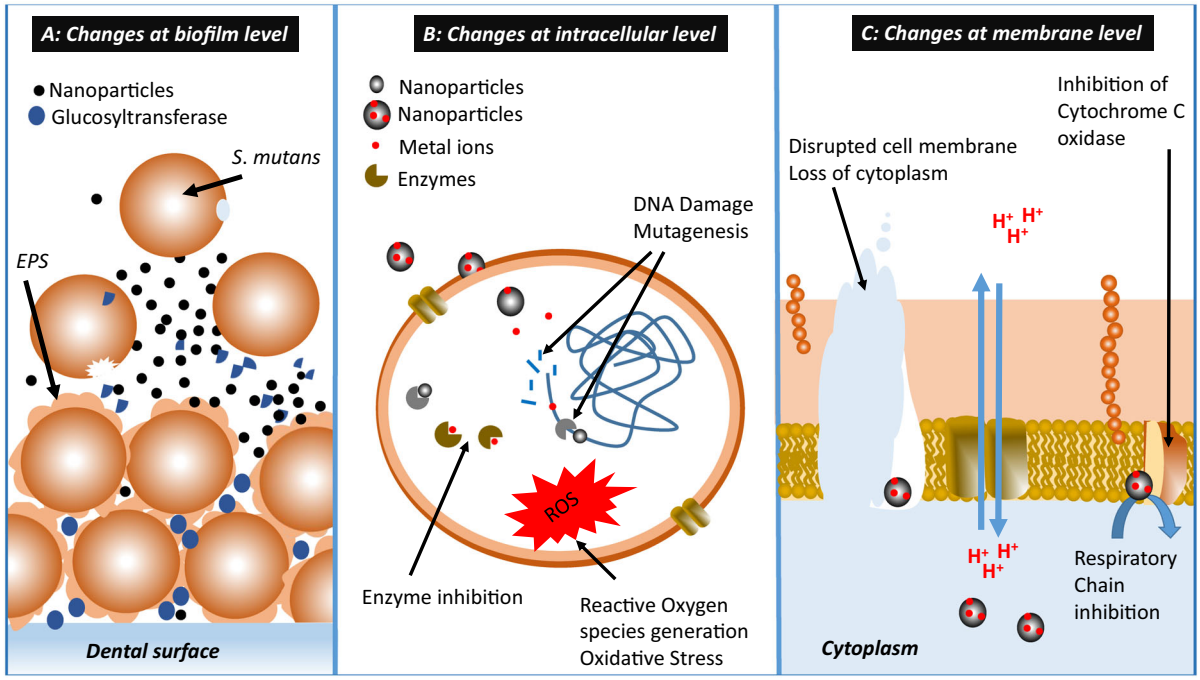

Fig. 3 Plausible mechanisms of NP-mediated antimicrobial and antibiofilm activities against oral bacteria. **a** *Shows* the inhibition of glucosyl transferase by NPs leading to the reduced exopolysaccharide production and biofilm formation. **b** *Shows* NP-mediated biochemical changes occurring at cellular level either in the planktonic cells or within the individual cells of the dental biofilm (e.g., ROS generation, DNA binding, enzyme inhibition). **c** *Shows* the changes taking place at the membranes of individual cells such as disruption of cell membrane, inhibition of cytochrome oxidase involved in the bacterial respiration

Bacillus subtilis are less susceptible to CuO NPs, a trend that corresponds with our findings on silver nanoparticles (Khan et al. 2014a). It has also been demonstrated that the fast-growing bacteria are more susceptible than slow-growing bacteria to antibiotics and nanoparticles (Brown et al. 1988; Sheng and Liu 2011). Most likely, the tolerance of slow-growing bacteria is related to the expression of stress response genes (Stewart 2002).

Several metals and metal oxides, carbon-based nanomaterials, and surfactant-based nanoemulsion have been reported to exhibit antibacterial activity (Huh and Kwon 2011; Li et al. 2008). However, the metallic NPs are regarded as promising candidates for overcoming bacterial resistance (Allaker 2010; Allaker and Memarzadeh 2014; Hajipour et al. 2012; Huh and Kwon 2011). Antimicrobial activity of NPs, including zinc oxide, silver, copper oxide, nickel, nickel oxide, tungsten trioxide, gold nanoparticles against oral bacteria, has been documented (Eshed et al. 2012; Espinosa-Cristóbal et al. 2012; Khan et al. 2013a, b; Lu et al. 2013), and is detailed in Table 2.

Metal oxide nanoparticles as potential antimicrobial agents with special reference to ZnO and TiO_2 NPs

Among the different types of NPs tested for antimicrobial activity, the Ag NPs and CuO NPs have been shown to exhibit excellent antimicrobial potential (Bondarenko et al. 2013). However, their global production is much lower than that of ZnO and TiO_2 NPs (Piccinno et al. 2012). ZnO NPs are the third largest produced NPs with a global annual production of ~550 tons per year (Piccinno et al. 2012, Keller et al. 2013). The production cost of ZnO NPs is also lesser than Ag NPs (Dastjerdi and Montazer 2010). Besides their use in cosmetics and paints (Piccinno et al. 2012), ZnO NPs are known for their excellent

Table 2 Metal and metal oxide nanoparticles tested for their antimicrobial activity against the pathogens and/or opportunistic pathogens of the oral cavity

Target Organisms	Nanoparticle	Size (nm)	MIC/MBC (μg ml^{-1})	Reference
Aggregatibacter actinomycetemcomitans	Ag NPs	5; 10–50	25 (MIC); 100 (MIC, MBC)	Lu et al. (2013), Vargas-Reus et al. (2012)
	CuO NPs	10–50	250 (MIC, MBC)	Vargas-Reus et al. (2012)
	Cu_2O NPs	10–50	<100 (MIC, MBC)	Vargas-Reus et al. (2012)
	TiO_2 NPs	10–50	1000 (MIC), >2500 (MBC)	Vargas-Reus et al. (2012)
	ZnO NPs	10–50	250 (MIC, MBC)	Vargas-Reus et al. (2012)
	WO3 NPs	10–50	2500 (MIC), >2500 (MBC)	Vargas-Reus et al. (2012)
Fusobacterium nucleatum	Ag NPs	10–50; 5	100 (MIC, MBC); 25 (MIC)	Vargas-Reus et al. (2012), Lu et al. (2013)
	CuO NPs	10–50	250 (MIC, MBC)	Vargas-Reus et al. (2012)
	Cu_2O NPs	10–50	<100 (MIC, MBC)	Vargas-Reus et al. (2012)
	TiO2 NPs	10–50	1000 (MIC), >2500 (MBC)	Vargas-Reus et al. (2012)
	ZnO NPs	10–50	250 (MIC), 500 (MBC)	Vargas-Reus et al. (2012)
	WO_3 NPs	10–50	2500 (MIC), >2500 (MBC)	Vargas-Reus et al. (2012)
Porphyromonas gingivalis	Ag NPs	10–50	250 (MIC, MBC)	Vargas-Reus et al. (2012)
	CuO NPs	10–50	500 (MIC), 2500 (MBC)	Vargas-Reus et al. (2012)
	Cu_2O NPs	10–50	100 (MIC, MBC)	Vargas-Reus et al. (2012)
	TiO_2 NPs	10–50	2500 (MIC), >2500 (MBC)	Vargas-Reus et al. (2012)
	ZnO NPs	10–50	250 (MIC, MBC)	Vargas-Reus et al. (2012)
	WO_3 NPs	10–50	2500 (MIC, MBC)	Vargas-Reus et al. (2012)
Prevotella intermedia	Ag NPs	10–50	100 (MIC, MBC)	Vargas-Reus et al. (2012)
	CuO NPs	10–50	250 (MIC, MBC)	Vargas-Reus et al. (2012)
	Cu_2O NPs	10–50	<100 (MIC, MBC)	Vargas-Reus et al. (2012)
	TiO_2 NPs	10–50	1000 (MIC), >2500 (MBC)	Vargas-Reus et al. (2012)
	ZnO NPs	10–50	1000 (MIC, MBC)	Vargas-Reus et al. (2012)
	WO_3 NPs	10–50	2500 (MIC), >2500 (MBC)	Vargas-Reus et al. (2012)
Streptococcus mitis	Ag NPs	5	25 (MIC)	Lu et al. (2013)
Streptococcus mutans	Ag NPs	5; 25; 9.3, 21.3, 98	50 (MIC); 4.86 (MIC), 6.25 (MBC); 64, 59.3, 13.2	Lu et al. (2013), Hernández-Sierra et al. (2008), Espinosa-Cristóbal et al. (2012)
	ZnO NPs	120–180;125	Biofilm inhibition; 500 (MIC, MBC)	Eshed et al. (2012), Hernández-Sierra et al. (2008)
	CuO NPs	18–20	Biofilm inhibition	Eshed et al. (2012)
	Au NPs	80	197 (bactericidal)	Hernández-Sierra et al. (2008)
	TiO_2 NPs	21	+antimicrobial activity	Konishi (1987)
Streptococcus sanguis	Ag NPs	5	50 (MIC)	Lu et al. (2013)
Rothia dentocariosa	ZnO NPs	35	53 (IC50)	Khan et al. (2014a, b)
Rothia mucilaginosa	ZnO NPs	35	76 (IC50)	Khan et al. (2014a, b)
Total oral bacteria	ZnO NPs	35	70 (EC50)	Khan et al. (2013a)
	CuO NPs	40	22 (EC50)	Khan et al. (2013a)
	Ni NPs	41.2	73 (IC50)	Khan et al. (2013b)
	NiO NPs	35.6	197 (IC50)	Khan et al. (2013b)

antimicrobial activity against pathogens (Allaker 2010; Allaker and Memarzadeh 2014; Jin et al. 2009; Liu et al. 2009). With regard to dental hygiene, ZnO NPs exhibited remarkable antimicrobial activity against a number of oral bacteria including *S. mutans* (Eshed et al. 2012), *Streptococcus sobrinus* (Aydin-Sevinç and Hanley 2010), *P.intermedia*, *P. gingivalis*, *Fusobacterium nucleatum, Aggregatibacter actinomycetemcomitans* (Vargas-Reus et al. 2012), *Rothia dentocariosa*, and *Rothia mucilaginosa* (Khan et al. 2014b). Both, the ZnO and TiO_2 are comparatively less toxic to humans than CuO and Ag NPs (Bondarenko et al. 2013; Yu and Li 2011, Sect. 8.0). Therefore, ZnO NPs and TiO_2 NPs are being utilized as drug carriers, in food packaging, cosmetics ingredients, and medical filling materials (Akbar and Kumar, 2014; Berube et al. 2010; Yu and Li 2011). Our earlier studies (Khan et al. 2013a, 2014b) suggest the possible application of ZnO NPs in oral pastes or mouth washes at concentration >100 μg/ml. Use of these nanoparticles in toothpastes is also reported by other authors (Yu and Li 2011; Vandebriel and De Jong 2012). ZnO NPs with an average size of 35 nm exhibit good antimicrobial activity against total oral bacteria at 70 μg/ml in vitro (Khan et al. 2013a; Fang et al. 2006). In addition to the desired antimicrobial and antibiofilm formation activities, ZnO NPs are also known to inhibit dentine demineralization (Takatsuka et al. 2005).

Several studies have suggested the inverse relationship between the size of NPs and their antimicrobial activity (Pal et al. 2007; Raghupathi et al. 2011; Lu et al. 2013; Adams et al. 2014). Khan et al. (2013a) demonstrated the IC_{50} value of 70.5 μg/ml with polygonal ZnO NPs (35 nm) against total oral bacteria. Aydin-Sevinç and Hanley (2010) reported the antimicrobial activity of 40–100 nm ZnO NPs against *S. sobrinus* with an MIC of 50 μg/ml against planktonic form of the bacteria. ZnO NPs with a size between 10 and 70 nm exhibited an MIC value of 250 μg/ml against *P. gingivalis*, *F. nucleatum*, *A. actinomycetemcomitans*, and an MIC value of 1.0 mg/ml against *P. intermedia* under anaerobic condition (Vargas-Reus et al. 2012). However, exposure to larger ZnO NPs with an average size of 125 nm has been shown to exhibit MIC/MBC value of 500 μg/ml against *S. mutans* (Hernández-Sierra et al. 2008), while still larger ZnO NPs (120–180 nm) do not inhibit the growth of *S. mutans* even up to a concentration of 1.0 mg/ml (Eshed et al. 2012). In addition to size, the shape of ZnO NPs also influences their antimicrobial activity. For instance, flower-shaped ZnO NPs with more sharp edges show higher antimicrobial activity against *E. coli* and *S. aureus* than relatively smoother rod- and sphere-shaped NPs (Talebiana et al. 2013). ZnO whiskers exhibited the MIC values of 78.1 and 312.5 μg/ml against *A. viscosus* and *S. mutans*, respectively (Fang et al. 2006).

One of the important factors that contributes in bacterial growth inhibition could be the release of zinc ions from ZnO NPs (Fukui et al. 2012). ZnO NPs also manifest their toxicity via generation of reactive oxygen species, such as hydrogen peroxide (Eshed et al. 2012). Leakage of intracellular content, cell wall, and membrane disruption by ZnO NPs in *E. coli* has been demonstrated using scanning electron microscopy and transmission electron microscopy (Liu et al. 2009). Also, the change in expression levels of genes involved in pathogenesis, oxidative stress responses, toxin production, and motility following exposure to ZnO NPs in *Campylobacter jejuni* has been studied (Xie et al. 2011). The expression of general stress response gene (*dna*K) and oxidative stress genes (*ahp*C and *kat*A) is reported to increase by 17-, 7-, and 52-fold, respectively, following the exposure to ZnO NPs, clearly demonstrating that ZnO NPs induced oxidative stress in bacteria. Cellular uptake of ZnO NPs in *E. coli* resulted in glutathione depletion and DNA damage (Kumar et al. 2011). However, most of these studies report only the MIC or IC_{50} values and the detailed studies of the possible interference of NPs with microbial processes resulting in their antimicrobial activities are lacking.

TiO_2 NPs are one of the most abundant NPs produced globally with some studies estimating a global production of 3000 tons per year (Piccinno et al. 2012; Keller et al. 2013). TiO_2 NPs are being extensively used even in food products; according to some findings, a typical US adult is already exposed to 1 mg/kg body weight per day of titanium, as it is used in a number of food products including chewing gums, candies and sweets with a code E171 (Weir et al. 2012). TiO_2 NPs demonstrate significant antimicrobial activity against a number of microorganisms including *E. coli*, *S. aureus*, *P. aeruginosa*, *E. faecium*, *B. subtilis*, and *Klebsiella pneumonia* (Rajakumar et al. 2012; Kühn et al. 2003). The MIC value of the TiO_2

NPs (62–74 nm) against these bacteria has been reported to be in the range of 40–80 μg/ml. However, the TiO_2 NPs with an average size of 21 nm exhibit an MIC value of 1 mg/ml against *S. sobrinus* (Saito et al. 1992). Konishi (1987) demonstrated the growth inhibition of oral bacteria including *S. mutans* HS-6 and *A. viscosus* ATCC 19246 by TiO_2 NPs at a concentration of 0.1 % (w/v). In another study, the mean MIC value of TiO_2 NPs against important oral biofilm-forming bacteria *P. intermedia*, *P. gingivalis*, *F. nucleatum*, and *A. actinomycetemcomitans* was found to be 1187.5 μg/ml (Vargas-Reus et al. 2012). The plausible mechanism of action of TiO_2 NPs against the bacteria could be ROS generation, DNA damage after internalization, peroxidation of membrane phospholipids, and inhibition of respiration (Kumar et al. 2011; Tsuang et al. 2008). Photoactivation of TiO_2 NPs also remarkably increased its antimicrobial activity against *Bacteroides fragilis*, *E. coli*, *E. hire*, *P. aeruginosa*, *S. typhimurium*, and *S. aureus* (Maness et al. 1999).

Nanoantibiotics and their antibiofilm activity with focus on ZnO and TiO_2

Biofilms are complex microbial communities adhered to solid surfaces by the secretion of extracellular matrix (containing extracellular polysaccharide, proteins, pili, flagella, adhesive fibers, and extracellular DNA), which cocoons the bacterial cell community. Bacteria in biofilms behave differently from their planktonic forms, forming complex 3D macroscopic structures containing channels and pores thus acting like multicellular organisms (Davey and O'Toole 2000; Costerton et al. 1995). These 3D, multicellular structures formed by pathogenic bacteria act as a protective shield against toxicants and antibiotics resulting in the development of chronic and recurring infections. Such biofilms exhibit significantly greater resistance to toxicants and antibiotic than the planktonic cells (Gilbert et al. 1997; Mah et al. 2003). Therefore, a good antibiofilm agent should have the ability to effectively penetrate through the biofilm in addition to possessing significant antimicrobial activity. In case of oral biofilms, an effective antiplaque agent should penetrate through the plaque and should reach enamel. Metal oxide NPs are emerging as propitious antimicrobial agents as discussed above. However, a few studies have demonstrated the antibiofilm activity of these nanoparticles and the molecular mechanism underlying their antibiofilm activity remains largely unexplained. Fabrega et al. (2011) demonstrated the inhibition of marine biofilm by Ag NPs, and reported a concentration-dependent reduction in biofilm formation. Although the mechanism of antibiofilm activity is not known, an important role of electrostatic attractions has been suggested. Positive charge of silver ions facilitates electrostatic attraction between the metal and the negatively charged bacterial membrane, augmenting uptake and antimicrobial activity (Kim et al. 2007). Ag^+ ions are known to inhibit DNA replication, expression of ribosomal subunit proteins, enzymes necessary for ATP production (Yamanaka et al. 2005), and membrane-bound respiratory enzymes (Bragg and Rainnie 1974). Antibiofilm activity of nitric oxide (NO)-releasing silica nanoparticles on the biofilms formed by *P. aeruginosa*, *E. coli*, *S. aureus*, and *S. epidermidis* has also been reported (Hetrick et al. 2009). The NO was shown to rapidly diffuse through the biofilms providing enhanced penetration resulting in the death of over 99 % cells from each type of biofilm. In a related study, considerable capability of magnetic NPs to penetrate into biofilms, using external magnetic fields, has been demonstrated (Park et al. 2011). ZnO NPs and CuO NPs when tested for the prevention of biofilm formation activity by a mixed oral bacterial population on artificial dental surfaces and on the surface of polystyrene plates show significant antibiofilm activity at concentrations lesser than 100 μg/ml (Khan et al. 2013a). A new class of multimodal NPs comprising a magnetic core and a silver ring with a ligand gap was engineered for the eradication of biofilm (Mahmoudi and Serpooshan 2012). These nanoparticles exhibited high antibacterial and antibiofilm activity and thus their use in theranosis has been proposed (Mahmoudi and Serpooshan 2012). Several studies on the coating of various surfaces such as glass, polyacrylic teeth, and catheters with nanoparticles with an aim to prevent and/or minimize the biofilm formation have been reported. Eshed et al. (2012) demonstrated 85 % reduction in biofilm formation activity of *S. mutans* on the surface of artificial teeth coated with ZnO NPs, as compared to control uncoated teeth. Coating of ZnO NPs on glass surfaces produces reactive oxygen species (ROS), which interferes with the *E. coli* and *S. aureus* biofilm formation (Applerot et al. 2012). ZnO NPs also show

marked inhibition of biofilm formation and hemolytic activity of *P. aeruginosa,* besides inhibition of pyocyanin, *Pseudomonas* quinolone signal (PQS), and pyochelin production. Transcriptome analyses of ZnO NPs exposed *P. aeruginosa* showed that ZnO nanoparticles induce the zinc cation efflux pump czc operon and several important transcriptional regulators including porin gene *opd*T and type III repressor *ptr*A (Lee et al. 2014). Comparative analysis of the inhibitory effect of Ni NPs (41 nm) and NiO NPs (35 nm) on biofilm formation activity of mixed oral bacteria revealed greater effect of Ni NPs compared to NiO NPs (Khan et al. 2013b). Engineered TiO_2 NPs impede the biofilm formation by *Shewanella oneidensis* (Maurer-Jones et al. 2013). However, another study shows that the coating of surface by TiO_2 does not affect the biofilm formation activity of two early colonizers of the oral cavity, namely *S. sanguinis* and *A. naeslundii* (Fröjd et al. 2011). Chun et al. (2007) demonstrated the bactericidal effect of TiO_2 NP-coated orthodontic wires on *S. mutans* and *P. gingivalis*, besides significant prevention of bacterial biomass deposition on their surface. Suketa et al. (2005) have suggested the photobactericidal effect of TiO_2 NPs layered metallic titanium on *A. actinomycetemcomitans* and *F. nucleatum,* with significant decrease in the viability of bacteria under UVA illumination within 120 min.

Hybrid nanocomposites and their antimicrobial activity

One of the most noticeable contributions of nanotechnology to oral hygiene and health is the nanoparticle-based dental materials with improved antimicrobial properties such as nanofillers, nanocomposites, and nanoparticle-based polymers (Cheng et al. 2012; Dwivedi et al. 2013; Hule and Pochan 2007). Development of such antimicrobial dental materials is a challenge as the addition of antimicrobial agents to these materials may adversely affect their physicochemical properties including hardness and mechanical strength (Hanemann and Szabó 2010). On the contrary, nanoparticle-based dental materials offer esthetic and strength advantages over conventional micro-filled and hybrid resin-based composite (RBC) systems besides possessing strong antimicrobial activity and remineralizing capabilities (Saunders 2009). These materials are also advantageous in terms of smoothness, polishability and precision of shade characterization, flexural strength, and micro-hardness, as compared to resin-based composites (Saunders 2009; Cheng et al. 2012).

Tavassoli-Hojati et al. (2013) developed resin composites containing various concentrations of ZnO NPs (0–5 wt%) and evaluated their physicochemical properties and antimicrobial activity against *S. mutans*. The antimicrobial activity of resins increased with the concentration of ZnO NPs incorporated without any change in the flexural strength and compressive modulus. On the contrary, compressive strength and flexural modulus of the resins improved significantly. Aydin-Sevinç and Hanley (2010) have also reported the synthesis of resins with different concentrations of ZnO NPs and suggested that ZnO NPs at a concentration of 10 % in these resins effectively inhibit the biofilm formation by *S. sobrinus*. The composite resins also showed significant inhibitory activity against the biofilm formed by *S. oralis*, *S. gordonii*, and *A. naeslundii* under anaerobic conditions (Aydin-Sevinç and Hanley 2010).

Incorporation of silver nanoparticles at a concentration of <1 % (w/v) in orthodontic bracket-bonding cement is reported to prevent the attachment and growth of the cariogenic bacterium *S. mutans*, without altering the physical properties of the cement (Allaker and Memarzadeh 2014). Similarly, silver complexes of poly(amidoamine) (PAMAM) dendrimers and silver-PAMAM dendrimer nanocomposite solutions have displayed considerable antimicrobial activity against *S. aureus*, *P. aeruginosa*, and *E. coli* (Balogh et al. 2001).

Nanotoxicology perspective

Indeed, there is a multitude of challenges in translating nanotechnology and nanoantibiotics, in particular, for clinical use. Toxicity of these NPs needs a careful and balanced evaluation before successful clinical translations. Key factors determining the toxicity of NPs include nature and extent of interactions of NPs with cells, tissues, and organs, and their proper routes of administration for desired therapeutic effects (Sandhiya et al. 2009; Suri et al. 2007). As the use of ZnO NPs and TiO_2 NPs in toothpastes and mouthwashes is proposed, the possibility of their ingestion warrants

the evaluation of their toxicity on intestinal epithelial cells both in vivo and in vitro. Exposure of RKO and Caco-2 human colon carcinoma cells to ZnO NPs with a size of 8–10 nm has been shown to yield changes in chaperonin proteins, metal metabolism, and protein-folding genes but did not show a pro-inflammatory signature (Moos et al. 2011). It has also been demonstrated in the same study that ZnO NPs (8–10 nm) and TiO_2 NPs (5 nm) both show minimal toxicity below 100 μg/cm^2. Exposure of the LoVo human colon carcinoma cell line to 11.5 μg/ml of ZnO NPs (50–70 nm) for 24 h resulted in decreased viability, increased H_2O_2/OH^-, decreased O_2^-, and glutathione depolarization of the inner mitochondrial membrane, apoptosis, and IL-8 release (De Berardis et al. 2010). Musarrat et al. (2009) also suggested the genotoxic potential of ZnO NPs (19.82 nm) at a higher concentration range of 100–400 μg/ml and their ability to perturb the mitochondrial membrane potential, possibly through oxidative mechanism on human lymphocytes. Bondarenko et al. (2013) in their review have compared the toxicity of 3 different NPs, namely Ag, CuO, and ZnO NPs. Based on 25 different in vitro studies on human cell lines, the median LE/LC_{50} value of ZnO NPs has been calculated to be 43 μg/ml, which is 4 times higher than that of silver (11.3 μg/ml), a well-known metal used in dentistry (Peng et al. 2012), which clearly shows that ZnO is safer than Ag NPs. Vandebriel and De Jong (2012) in their review of mammalian toxicity of ZnO NPs have concluded that genotoxicity of ZnO NPs was only observed in in vitro and not in vivo studies and the toxicity in in vitro assays was largely due to the oxidative stress. Warheit et al. (2007) investigated in vivo and in vitro toxicity of ultrafine TiO_2 (140 nm) and concluded that this form of oxide exhibited low hazard potential in aquatic and mammalian species/cell lines following acute exposure. In another in vivo study on terrestrial isopods Valant et al. (2012) found that ingestion of TiO_2 nanoparticles exhibits toxic effects only at a high concentration of 1000–2000 μg/g of feed.

Thus, the proposed application of ZnO NPs and TiO_2 NPs in toothpastes and mouthwashes, as supplementary antibacterial agents, may not exert the acute adverse effects to human cells, due to short exposure time and at proposed non-toxic doses (>100 μg/ml). Nevertheless, it remains crucial to determine the actual doses of the nano-based formulations, to which the oral cavity is exposed, and possibly reach the gastrointestinal tract by accidental ingestion, and to assess the in vivo toxicity of these doses.

Conclusion

The increasing evidence of the involvement of oral bacteria in a number of systemic diseases and the development of antibiotic resistance among oral bacteria is a matter of serious concern. Metal oxide nanoparticles especially ZnO and TiO_2 NPs exhibit good antimicrobial activity against the oral bacteria, and therefore offer a good alternative for traditional antibiotics. However, their applications in oral hygiene are still in infancy. Therefore, systematic and mechanistic studies are required to understand the impact of these NPs in combating the oral infections and biofilm formation.

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