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Application of nanoparticles in oral hygiene

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ABSTRACT

Many of more than 700 bacterial species inhabiting the oral cavity are opportunistic pathogens causing systemic infections in addition to dental and periodontal diseases. This renders oral hygiene a much serious issue, which is further exacerbated with the emergence of multiple antibiotic resistance in oral bacteria. The role of nanoparticles based materials especially metal and metal oxide nanoparticles as an effective and alternative/supplementary antimicrobial agent is now well established. These nanoparticles could be a healthier, innocuous and effective alternative for controlling both the dental biofilms and oral planktonic bacterial population with lesser side effects or antibiotic resistance. Antimicrobial activity of these nanoparticles against a number of oral pathogens has already been demonstrated. When added to artificial dental materials and implants these nanoparticles improve the desirable physico-chemical properties of the materials in addition to improving their antimicrobial activity. Besides a few studies, biochemical processes underlying the antimicrobial activity of the nanoparticles against both planktonic cells and oral biofilms is not understood. Through our literature survey it is envisaged that ZnO nanoparticles and TiO_2 nanoparticles are the most suitable nanoantibiotic for the development of dental pastes, mouthwashes, and other oral hygiene materials. However in vivo studies on nanotoxicity of these nanoparticles are missing and need a careful and balanced evaluation before successful clinical translations.

Keywords: *Nanostructures, Oral Hygiene, Bacteria, Toxicology, Pharmacology.*

1. INTRODUCTION

Human oral microbiome is one of the most complex microbiomes, different studies estimate the presence of 25,000 different phylotypes or 700 different species of bacteria in the oral cavity (Jenkinson et al. 2011; Keijser et al. 2008; Liu et al. 2012; Paster et al. 2006).

This remarkable diversity of the microbial community can be attributed to the diverse micro- niches within the oral cavity, distinguished by their physico-chemical properties (Segata et al., 2012). The teeth, tongue, mucosa, palate, and gingiva harbor distinctive microbiota as found by both culture dependent and culture independent approaches (Aas et al., 2005; Jenkinson et al., 2011; Keijser et al. 2008; Liu et al., 2012; Paster et al., 2006). It is often difficult to culture all the microorganisms present in a niche due to the diverse and often unknown nutritional and physico-chemical requirements of the diverse bacteria present in a given niche.

Molecular-based, culture-independent techniques, such as pyrosequencing, and 16S rRNA profiling, have provided important new insights into the diversity of the microbiome within the oral cavity (Crielaard et al., 2011). Especially pyrosequencing allows an extensive and high-throughput characterization of microbial communities. In an interesting study using pyrosequencing it was observed that microbial community present even on two different sites of the same tooth varied significantly. For instance, the genera Streptococcus constituted 29% to 70% on the vestibular surfaces of teeth but on the suculus side constituted only 5% to 21% in almost all the samples studied. While quadrants 1 and 2, displayed a higher percentage of Aggregatibacter and Capnocytophaga. This, diversity of microbial populations can be attributed to the heterogeneity of physicochemical properties of the micro-niches within the oral environments. Even the same tooth or teeth in close proximity were found to have different pH, oxygen levels, temperature, and redox potential (Kleinberg and Jenkins, 1964). These variations in physico chemical characteristics of micro-niches influence the colonization of micro-organisms (Fejerskov et al., 1994). Despite this diversity, a number of investigators have tried to identify bacteria involved in the oral diseases and to distinguish them from normal oral bacteria (Aas et al., 2005).

However, the biochemical conditions favoring the proliferation of pathogenic bacteria within the oral cavity leads to periodontitis, an inflammatory disease, which can also constitute a risk factor for other systemic diseases (Zbinden et al., 2012) such as endocarditis and colorectal cancer (Han et al., 2013). Therefore, it is both urgent and important to clarify the role of microbial communities in systemic diseases and human health (Belda-Ferre et al., 2011; Turnbaugh et al., 2007; Ximénez-Fyvie et al., 2000). The problem is further complicated by the development of resistance to traditional antibiotic in oral bacteria, which is not only limited to the human subjects receiving antibiotic therapies (Leistevuo et al., 2000; Sweeney et al., 2004).

The search for new, effective and economic alternative antimicrobial agents is therefore crucial to combat microbial infections. Metal oxide nanoparticles exhibit remarkable antimicrobial activity and are also referred to as nanoantibiotics (Huh and Kwon, 2011). Hence, the role of these nanoantibiotics to abate and/or control the growth of bacteria in oral cavity (Allaker, 2010) is proposed here. In this review, the impact of oral cavity

bacteria on oral and other systemic diseases is discussed briefly, with an emphasis on the role and mechanism of metal oxide nanoparticles in impeding the growth and biofilm formation activity of oral bacterial.

2. HEALTH RISK ASSOCIATED WITH ORAL BACTERIA

Dental and periodontal diseases are one of the most common oral diseases, it was estimated by Centers for Disease Control (CDC) USA that in years 2009 and 2010, 47% of 64.7 million American adults suffered from mild to severe dental ailments. While the prevalence in adults 65 years and older was as high as 70% (Eke et al., 2012). The annual global monetary loss due to the dental diseases is estimated to be 442 billion US dollars, which is 4.6% of global health expenditures. Moreover, if losses due to various systematic infections and diseases culminating from recurrent oral infections are to be considered, then this issue is much more complicated. Regardless of the high social and financial burden of oral diseases they are considered as neglected area of international health.

Oral cavity is one of the most densely populated regions, where the microorganisms colonize at the oral surfaces, and develop a microbial consortium, in the form of dental plaque or oral biofilm. Amongst the consortia, Streptococcus mutans and Porphyromonas gingivalis have received considerable attention due to their role in dental caries and periodontitis, respectively. However, the heterogeneity of tissue types in the oral cavity, such as teeth, tongue and mucosa, provides specific ecological niches for microbial colonization resulting in supragingival plaque, subgingival plaque and tongue coating.

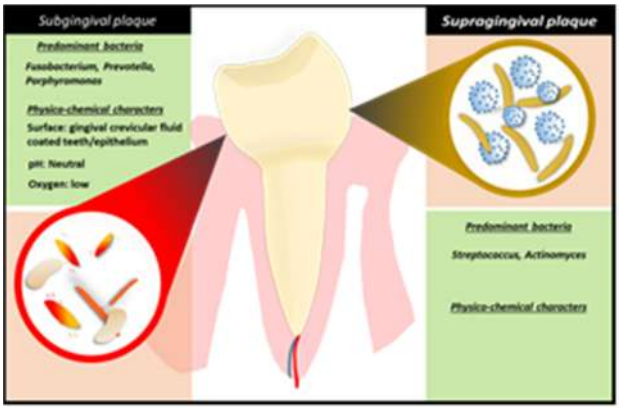

Figure 1. Physico-chemical characteristics of ecological niches within the oral cavity supporting niche specific microorganisms (Modified from Khan et al., 2015).

Each of these niches can be characterized based on the environmental factors and metabolic characteristics of the resident microbial flora (Figure 1; Takahashi 2005).

2.1. Dental caries, periodontal diseases and microbes.

Dental caries or teeth decay is demineralization and destruction of hard tissues of the teeth, which can progress and cause inflammation and destruction of surrounding soft tissues, ultimately resulting in periodontitis. Both, dental caries and periodontal diseases have multiple aetiologies, but are largely caused by bacteria. Oral microbiome is one of the most extensively studied human microbiome both for normal and diseased subjects (Nasidze et al., 2009; Wang et al., 2013). Bacterial species belonging to 13 different phyla namely Actinobacteria, Bacteroidetes, Chloroflexi, Firmicutes, Fusobacteria, Proteobacteria, Spirochaetes, Synergistetes, Tenericutes, and two currently unnamed phyla, SR1 and TM7, inhabit the oral cavity (Aas et al. 2005; Dewhirst et al. 2010; Munson et al. 2004; Paster et al. 2001). Some of these phyla are found in all the humans yet high diversity of salivary microbiome within and between individuals has 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, wherein the bacteria that initiate biofilm formation attach firmly and irreversibly on the surface of the teeth by evading the host defense system, and provide a surface for the attachment of more acidogenic secondary colonizers. Although, S. mutans has been identified as one of the most important organisms causing dental caries back in 1924 (Clarke, 1924), but the polymicrobial nature of the disease is now very well established (Darveau, 2010). Socransky et al. (1998) defined the red complex of bacteria consisting of Treponema denticola, P. gingivalis, and Tannerella forsythia and their collective ability to interfere with host defense mechanism, and hence found to be associated with diseased site. Among the primary colonizers that initiate the biofilm formation are S. oralis, S. mitis, S. sanguinis, S. parasanguinis and S. gordonii, Actinomyces, Veillonella, Gemella, Abiotrophia, Granulicatella, and Lactobacillus (Darveau, 2010; Hojo et al., 2009; Huang 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, proteases like immunoglobulin A1 (IgA1 protease) (Cole et al. 1994), and glycosidases in addition to the substances required for their binding on the surface of saliva coated teeth such as exopolysaccharides, flagella, environmental DNA (eDNA), proteins and lipoproteins. S. gordonii binds to salivary agglutinin glycoproteins by SspA/B, to alpha-amylase by AbpA, and to the α2-3-linked sialic acid termini of O-linked oligosaccharides of host glycoconjugates by HAS (Kolenbrander et al., 2002). Aggregatibacter actinomycetemcomitans produces a leucotoxin that specifically lyses human neutrophils, the first line of innate host defense. Furthermore, 50% of the Streptococci isolates were found to produce immunoglobulin A1 (IgA1) protease and glycosidases (Bradshaw et al., 2004; Frandsen et al. 1986; Haubek et al., 2004). In another study, Streptococcus has been shown to produce IL-8 protease, which promotes resistance to neutrophil killing (Zinkernagel et al., 2008). 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 in an in vivo study (Skopek et al., 1993). A total of 250,000 bacteria/mm2 colonize the surface of a preformed biofilm

within a few hours of exposure. Among secondary colonizers are P. gingivalis, P. intermedia, T. denticola, F. nucleatum, and A. actinomycetmcomitans and Lactobacillus (Figure 2). Many of these microorganisms in an established biofilm support the mutual growth and biofilm formation (Huang et al., 2011). The biofilm utilizes dietary sugars especially sucrose and continuously produce acids causing demineralization of enamel and bone loss.

Clinical observations revealed that carries and periodontal diseases have a chronic-cumulative character and no continuous way of progression (Gaengler et al. 2009).

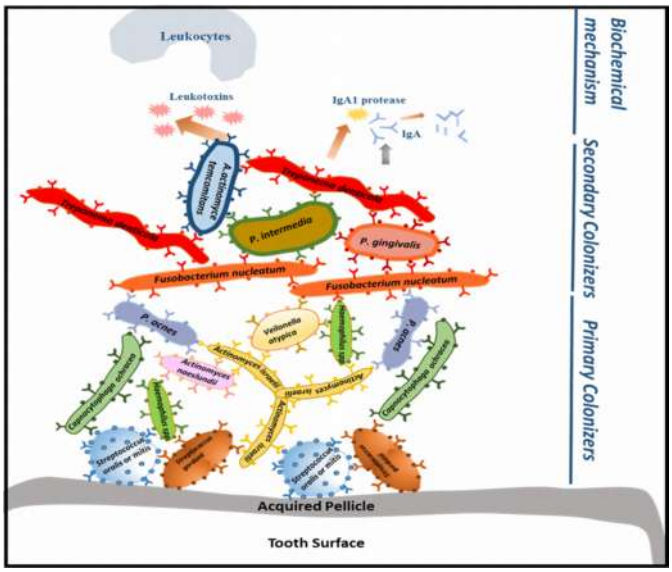

Figure 2. Biofilm formation by oral bacteria on the surface of the teeth (Modified from Khan et al., 2015).

Indeed, periodontal disease is the most common infectious disease affecting tooth-supporting structures. If remains unattended, it may aggravate various systemic diseases (Ali et al., 2011; Bascones-Martinez et al. 2011; Teles and Wang, 2011).

2.2. Systemic diseases caused by oral bacteria.

Therefore, it is crucial to understand the dynamics of oral microbiome for early diagnosis and treatment before the clinical manifestations. Also, the emergence and evolution of antibiotic resistance in periodontal pathogens has affected the therapeutic success rates for this disease (Rodrigues et al., 2004; van Winkelhoff et al., 1999). Unravelling the complex interactions that define the oral microbiome and development of new approaches are urgently needed to help regain control over periodontal disease.

Oral infections, specifically periodontitis, leads to the progression and pathogenesis of many systemic diseases, including the diabetes mellitus, cardiovascular disease, bacterial pneumonia and low birth weight (Li et al. 2000). Hence, for good general health it is crucial to maintaining good oral health and vice versa. 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 in a number of ways, such as (i) transient bacteremia resulting in the spread of metastatic infections, (ii) circulating oral microbial toxins causing metastatic injury, and (iii) oral microorganisms induced immunological injuries causing metastatic inflammation (Li et al., 2000). Subgingival biofilms with enormous bacterial load serves as reservoirs of LPS and other Gram-negative bacteria with ready access to the periodontal tissues and the circulation. It has been suggested that the vascular responses such as inflammatory cell infiltration in the vessel walls, vascular smooth muscle proliferation, vascular fatty acid degeneration, and intravascular coagulation are induced by Gram-negative bacteria and/or LPS (Marcus and Hajjar, 1993; Mattila, 1999; Williams and Offenbacher, 2000). LPS also up-regulates expression of endothelial cell adhesion molecules and secretion of interleukin-1 (IL-1), tumor necrosis factor alpha (TNF-α), and which results in platelet aggregation and adhesion, thromboxane and formation of lipid-laden foam cells, and deposits of cholesterol and cholesterol esters (Herzberg and Meyer 1996). The proinflammatory cytokines IL-1β, TNF-α, and gamma interferon as well as prostaglandin E2 (PGE2) reach high tissue concentrations in periodontitis (Page, 1998). These mediators in circulation have also shown to cause preterm labor and low-birth-weight infants (Han et al., 2010).

F. nucleatum one of the predominant bacteria involved in adverse pregnancy outcomes have been isolated from foetal membranes, amniotic fluid, cord blood, neonatal gastric aspirates, foetal lung and stomach (Han et al., 2010; Han and Wang, 2012). It has also been demonstrated that the F. nucleatum originated from mother's subgingival plaque (Han et al., 2010; Han and Wang, 2012). 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 cells through FadA adhesin and invade these cells.

FadA binds to VE-cadherin receptors on the surface of endothelial cells increasing their permeability thus allowing the penetration of other bacteria into the cells (Fardini et al., 2011). After penetration into the cells, 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). The heat shock protein GroEL of F. nucleatum induces factors that predispose to atherosclerosis in human microvascular endothelial cells (HMEC-1). Injection of this protein in apolipoprotein E-deficient mice shows significant progression of atherosclerotic lesion than in control mice (Lee et al., 2012). Several subgingival bacteria such as A. actinomycetemcomitans (Yuan et al., 1994), Actinomyces israelii (Morris and Sewell 1994), Capnocytophaga spp. (Lorenz and Weiss, 1994), Eikenella corrodens (Joshi et al., 1991), Prevotella intermedia, and Streptococcus constellatus (Shinzato and Saito, 1994) have been implicated in pneumonia.

The role of oral bacteria in cardiovascular disease is now undoubtedly clear as several mechanisms by which periodontal disease may trigger pathways leading to cardiovascular disease are now proposed. Herzberg and Meyer (1996) provided the first evidence that oral bacteria S. sanguis and P. gingivalis induce platelet aggregation leading to thrombus formation. These bacteria have a collagen-like platelet aggregation-associated protein on their surface (Herzberg et al., 1994). Herzberg and Meyer (1996)

also demonstrated that S. sanguis injected intravenously into rabbits, triggers a heart attack-like series of events. Most likely, antibodies reactive to periodontal organisms gets localized in the heart and induce complement activation leading to sensitized T cells and heart disease.

Another important bacterium involved in a number of systemic diseases is P. gingivalis. Deshpande et al. (1998) have suggested that P. gingivalis can actively adhere to and invade foetal bovine heart endothelial cells, bovine aortic endothelial cells, and human umbilical vein endothelial cells. Its mechanism of pathogenicity and involvement in cardiovascular disease and atherosclerosis has been comprehensively reviewed (Hayashi et al., 2010). Furthermore, LPS in serum from invaded periodontal microorganisms exerts a direct effect on endothelia, which may promote atherosclerosis (Pesonen et al., 1981).

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 manifest its pathogenicity is peptidylarginine deiminase (PAD).

PAD found only in very few prokaryotes catalyzes protein citrullination, which has been linked to rheumatoid arthritis (Maresz et al., 2013).

Table 1. Systemic diseases due to 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. (2013)
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)

3. ANTIMICROBIAL RESISTANCE IN ORAL BACTERIA

Growing resistance to traditional antibiotics in pathogenic bacteria at pace much faster than the discovery of new ones is one of the most serious health challenges of our time. CDC estimates that more than two million people are infected by antibiotic-resistant bacteria and 23,000 subsequently die in United States alone per year. WHO estimates that multi-drug resistant tuberculosis alone caused about 170,000 deaths in 2012. The failure to discover new antibiotics is evident from the fact that it took almost 30 years to discover a new antibiotic Teixobactin. Oral bacteria are also developing resistance to the traditionally used antibiotics (Ready et al., 2003). Some of the commonly used antibiotics used for treating dental and oral infections include penicillin V, erythromycin, amoxicillin and metronidazole constituting 60, 14, 12, and 8% of total prescriptions, respectively (Ready et al., 2003). Resistance to these antibiotics is widespread among oral bacteria even in the children with no previous exposure to these antibiotics (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 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 blaTEM (17%), followed by tetracycline tetW (10%), and macrolide erythromycin ermC (10%). The prevalence of tetQ (tetracycline), cepA (β-lactamase) and cblA (β-lactamase) genes has also been documented (Kirchner et al. 2013). Interestingly, both the erm(B)

gene is and tet(M) genes are found on a highly mobile conjugative transposon Tn1545, found predominately present in clinically important Gram-positive bacteria.

The presence of glycopeptide and macrolide resistance genes on the same transferable genetic element in Enterococcus faecium have also been described from pigs and humans. It is also well known fact that 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.

4. COMBATING ANTIBIOTIC RESISTANCE BY NANOANTIBIOTICS

Project on emerging nanotechnologies has reported about 1824 commercial products that are currently using engineered nanomaterials including biomedicine (Berube et al., 2010). Plethora of biomedical applications has also been proposed for nanoparticles based on their unique physico-chemical properties. Their role in hyperthermia treatment of cancer (Banobre-López et al., 2013), biosensors (Luo et al., 2006), surgery (Ou et al., 2014), therapeutics (Zhang et al., 2008) imaging (Drummen, 2010), 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. Nanobased 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 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. Nanoparticles can 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., 2012a).

Mechanisms underlying the antimicrobial activity of nanomaterials include (i) disruption of bacterial cell membrane (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 arise 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 Bacillus subtilis are less susceptible to CuO NPs, a trend that corresponds with our findings on silver and cobalt nanoparticles (Khan et al., 2014a, 2015). 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).

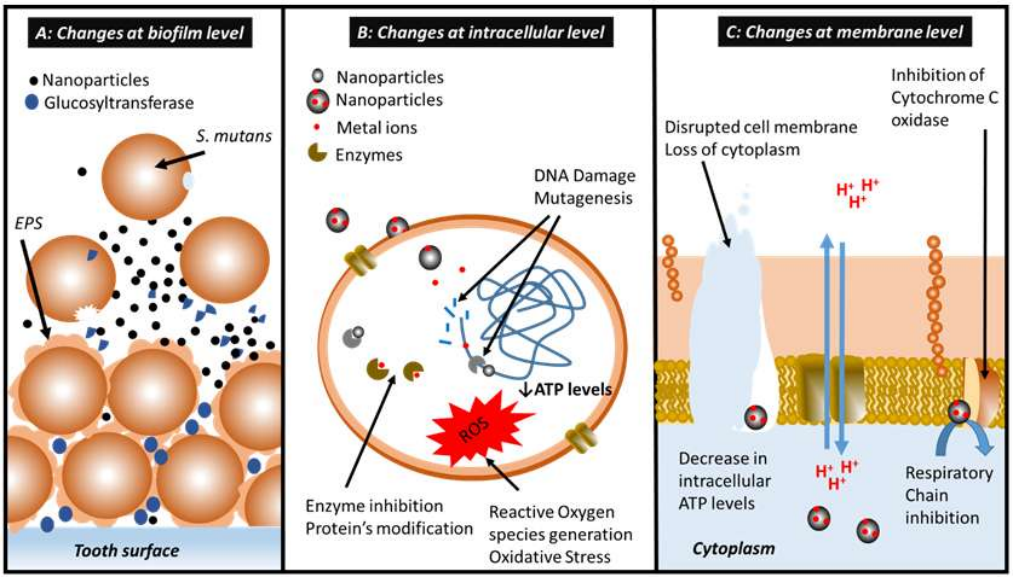

Figure 3. Mechanisms of NP mediated antimicrobial and antibiofilm activities against oral bacteria. Panel A shows the inhibition of glucosyl transferase by NPs leading to the reduced exopolysaccharide production and biofilm formation and loosening of biofilm through penetration. Panel B shows NPs mediated 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, depleted levels of intracellular ATP). Panel 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 (Modified from Khan et al., 2015).

5. ANTIMICROBIAL ACTIVITY OF METAL AND METAL OXIDE NANOPARTICLES AGAINST ORAL BACTERIA

Metals and metal oxides, carbon-based nanomaterials, and surfactant-based nanoemulsion exhibit excellent antibacterial activity (Huh and Kwon 2011; Li et al., 2008). However, metal and metal oxide 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, Khan et al., 2013b; Lu et al,. 2013), and is detailed in Table 2. The antimicrobial activity of some metal and metal oxide nanoparticles against oral bacteria is discussed below.

Table 2. Metal and metal oxide nanoparticles tested for their antimicrobial activity against the oral pathogens and/or opportunistic pathogens of the oral cavity (Adopted from Khan et al., 2015).

Target Organisms	Nanoparticle	Size (nm)	MIC/MBC (μg ml^{-1})	Reference
Aggregatibacter actinomycetemcomitans	Ag	5; 10-50	25 (MIC); 100 (MIC, MBC)	Lu et al., 2013; Vargas-Reus et al., 2012
	CuO	10-50	250 (MIC, MBC)	Vargas-Reus et al., 2012
	Cu_2O	10-50	<100 (MIC, MBC)	Vargas-Reus et al., 2012
	TiO_2	10-50	1000 (MIC), >2500 (MBC)	Vargas-Reus et al., 2012
	ZnO	10-50	250 (MIC, MBC)	Vargas-Reus et al., 2012
	WO_3	10-50	2500 (MIC), >2500 (MBC)	Vargas-Reus et al., 2012
Fusobacterium nuceatum	Ag	10-50; 5	100 (MIC, MBC); 25 (MIC)	Vargas-Reus et al., 2012; Lu et al., 2013
	CuO	10-50	250 (MIC, MBC)	Vargas-Reus et al., 2012
	Cu_2O	10-50	<100 (MIC, MBC)	Vargas-Reus et al., 2012
	TiO_2	10-50	1000 (MIC), >2500 (MBC)	Vargas-Reus et al., 2012
	ZnO	10-50	250 (MIC), 500 (MBC)	Vargas-Reus et al., 2012
	WO_3	10-50	2500 (MIC), >2500 (MBC)	Vargas-Reus et al., 2012
Porphyromonas gingivalis	Ag	10-50	250 (MIC, MBC)	Vargas-Reus et al., 2012
	CuO	10-50	500 (MIC), 2500 (MBC)	Vargas-Reus et al., 2012
	Cu_2O	10-50	100 (MIC, MBC)	Vargas-Reus et al., 2012
	TiO_2	10-50	2500 (MIC), >2500 (MBC)	Vargas-Reus et al., 2012
	ZnO	10-50	250 (MIC, MBC)	Vargas-Reus et al., 2012
	WO_3	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	5	25 (MIC)	Lu et al., 2013
	Ag	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., ,2013
Streptococcus mutans	ZnO	120-180;125	biofilm inhibition; 500 (MIC, MBC)	Eshed et al., 2012; Hernández-Sierra et al., 2008
	CuO	18-20	biofilm inhibition	Eshed et al., 2012
Streptococcus sanguis *Rothia dentocariosa* *Rothia mucilaginosa*	Au	80	197 (bactericidal)	Hernández-Sierra et al., 2008
	TiO_2	21	+ antimicrobial activity	Konishi 1985
	SiO_2	500-1000	no effect	Besinis et al., 2014
	Ag	<100	50 (MIC)	Besinis et al., 2014
	Ag	5	50 (MIC)	Lu et al., 2013
	ZnO	35	53 (IC_{50})	Khan et al., 2014
	ZnO	35	76 (IC_{50})	Khan et al., 2014
Total oral bacteria	ZnO	35	70 (EC_{50})	Khan et al., 2013a
	CuO	40	22 (EC_{50})	Khan et al., 2013a
	Ni	41.2	73 (IC_{50})	Khan et al., 2013b
	NiO	35.6	197 (IC_{50})	Khan et al., 2013b

5.1. Silver nanoparticles.

Despite the toxicity concerns silver is a widely used metal in dentistry (Peng et al., 2012; Lansdown, 2006). Although the global production of silver nanoparticles is about 10 times lower than SiO2 and ZnO nanoparticles its antimicrobial activities are much more widely reported including against oral bacteria (Khan et al., 2014; Lu et al., 2013; Vargas-Reus et al., 2012). Interestingly even the multidrug resistant bacteria show sensitivity to Ag NPs at a concentration as low as 20 μg/ml (Molecules, 2015, 20, 8856-8874). Bactericidal activity of silver nanoparticles against a number of oral bacteria including S. mutans and F. nucleatum is reported in various studies and is summarized in Table 1. Silver results in bacterial mortality by binding to sulfhydryl groups of proteins and with DNA, adversely affecting, respiratory processes, cell division, cell-wall synthesis, metabolism of purine, destabilizing the outer membrane, and depletes the intracellular levels of ATP (Cui et al., Lansdown, 2006; Lok et al., 2006; Rahban et al., 2010). It has been proved that the antimicrobial activity of Ag NPs depend on both size and shape of the NPs. Lu et al. (2013) has demonstrated that the antimicrobial activity of Ag NPs depends on their size. In their study the smallest nanoparticle with a size of 5 nm was most effective against all the tested oral pathogens (A. actinomycetemcomitans, F. nuceatum, S. mitis, S. mutans and S. sanguis).

In another interesting study, it was proved that Ag-nanomaterials with shapes (truncated triangular silver nanoplates) capable of inflicting greater mechanical injuries to bacteria show

increased biocidal activity (Pal et al., 2007). Release of silver ions from Ag NPs also contribute to their microbicidal activity. In a study on E. coli it has been demonstrated that silver ions inhibited the oxidation of fumarate, glucose, glycerol, lactate and succinate, and other endogenous substrates.

Silver ions also interfered with respiratory processes by inhibition of 6-cytochromes and cytochrome 2, and the inhibition of succinate dehydrogenase (Bragg and Rainnie, 1974). Binding of silver ions to various biomolecules as explained above can also force the bacteria to enter into an active but non culturable state (ABNC; Jung et al., 2008) ultimately leading to death.

On the contrary, some studies argue that the amount of silver ions released from the nanomaterials is very low and hence the antimicrobial activity of silver NPs is largely due to their nano shape and not silver ions released therefrom (Lok et al., 2006).

5.2. Zinc oxide nanoparticles.

Zinc oxide nanoparticles are one of the most suitable nanoparticles for oral hygiene as discussed by Khan et al. (2015b), with third largest global production of ~550 tons per year (Piccinno et al. 2012, Keller et al. 2013). Moreover, ZnO NPs are cheaper than Ag NPs (Dastjerdi and Montazer 2010), exhibit excellent antimicrobial activity against pathogens (Allaker, 2010; Allaker and Memarzadeh, 2014; Jin et al., 2009; Liu et al., 2009), and are comparatively less toxic to humans than CuO and Ag NPs (Bondarenko et al., 2013; Yu and Li, 2011).

With regard to dental hygiene, ZnO NPs exhibited remarkable biocidal activity against a number of oral bacteria including Streptococcus mutans (Eshed et al., 2012), Streptococcus sobrinus (Aydin-Sevinç and Hanley, 2010), Prevotella intermedia, Porphyromonas gingivalis, Fusobacterium nucleatum, Aggregatibacter actinomycetemcomitans (Vargas-Reus et al., 2012), Rothia dentocariosa and Rothia mucilaginosa (Khan et al., 2014b).

Several studies have suggested the inverse relationship between the size of ZnO 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 IC50 value of 70.5 µg/ml with polygonal ZnO NPs (35nm) 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 10-70 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 have 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 influence 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).

Another important factor contributing to the antimicrobial activity of ZnO NPs is 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).

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 (dnaK) and oxidative stress genes (ahpC and katA) increases by 17, 7 and 52 folds, respectively following the exposure to ZnO NPs, clearly demonstrating the ZnO NPs mediated induction of oxidative stress in bacteria.

However, most of these studies report only the MIC or IC50 values and the detailed studies of the possible interference of NPs with microbial processes resulting in their antimicrobial activities are lacking.

5.3. TiO_2 nanoparticles.

TiO_2 NPs, are one of the most abundant NPs produced globally with an estimated 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 estimates 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 1mg/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).

5.4. Copper oxide (CuO) nanoparticles.

CuO also show remarkable broad spectrum antimicrobial activity against a number of bacteria including MRSA (Ren et al., 2009). Antimicrobial activity of CuO NPs against oral pathogens is summarized in table 1. The MIC values of CuO (10-50 nm) against the oral bacteria ranged between 250-500 µg/ml against a number of important oral pathogen such as S. mutans, F. nuceatum and P. gingivalis (Table 1). As far as the mechanisms of its antimicrobial activity are concerned induction of reactive oxygen species by Cu2+ released from CuO NPs has been demonstrated consequently damaging DNA and inducing mutations (Pan et al., 2010). Inhibition of cellular respiration in E. coli by CuO NPs was also reported by Wahab et al., 2013. For CuO NPs also it has been argued that the Cu2+ ions dissolved from CuO NPs have very little effect on the antibacterial activity of the NPs (Applerot et al., 2012b). In fact, the ROS generation is due to the CuO NPs and not due to the Cu2+ ions. Strong binding of the CuO nanoparticles to the bacterial cell membrane, and subsequent ROS generation results in increased cell permeability of nanoparticles. And this unchecked penetration of NPs into the bacterial cells ultimately leads to cells death. CuO nanoparticles also induce lipid peroxidation and deplete intracellular levels of ATP (Applerot et al., 2012b). Like other nanoparticles the antimicrobial activity of CuO NPs also depend on their shape and size (Azam et al., 2012). Moreover, like other nanoparticles CuO NPs also show higher antimicrobial activity against Gram positive bacteria than Gram negative bacteria (Azam et al., 2012; Wahab et al., 2013).

5.5. Other nanoparticles.

Bactericidal activity of other nanoparticles including gold, tungsten, nickel and nickel oxide nanoparticles against oral pathogens and opportunistic pathogens has been demonstrated details of some of these nanoparticles is listed in table 1. Nickel mediated inhibition of total oral bacteria and its antibiofilm activity has been reported (Khan et al., 2014). Nickel is a metal traditionally used in dentistry despite the toxicity concerns (Arikan 1992).

6. ANTIBIOFILM ACTIVITY OF METAL AND OXIDE NANOPARTICLES

The accumulation of bacterial biofilms or plaques on the surface of tooth is one of the most important causes of dental caries and periodontal diseases. Therefore, it is of immense clinical importance to check the biofilm formation on the oral surfaces. Biofilms are complex microbial communities adhered to solid surfaces by secretion of extracellular matrix (containing extracellular polysaccharide, proteins, pili, flagella, adhesive fibers and extracellular DNA), cocooning the bacterial cell community. Bacteria in biofilms behave differently from their planktonic forms, forming complex 3-D 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 and are difficult to treat (Gilbert et al., 1997; Mah et al., 2003). This increased resistance of biofilms is due to the following properties of the biofilms. High density of cells attached to solid surfaces in a biofilm results in increased metabolic efficiency. This high cell density and increased metabolic activity facilitates the evasion of host-defenses, by promoting events such as horizontal gene transfer against antimicrobial agents. Furthermore, biofilms also serve as reservoirs of microbial cells that can detach migrate and colonize other sites. Therefore, an effective antiplaque agent should disrupt existing biofilm and should prevent new biofilm formation in addition to killing particular organisms actively involved in biofilm formation.

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, but 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). Inhibition of Pseudomonas putida biofilms by Ag NPs (60 nm) was also reported earlier. Kalishwaralal et al. (81) also reported the antibiofilm activity of Ag NPs against two keratitis causing organisms (P. aeruginosa and S. epidermidis). Ag NPs show 95-98% decrease in biofilms following a short treatment of 2h with Ag NPs. Three folds higher reduction in biofilms with Ag NPs was observed when the ability of Ag NPs to disrupt biofilms of P. aeruginosa was compared with the antibiofilm activity of LL-37, a peptide known to impair biofilm formation.

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 of a magnetic core and a silver ring with a ligand gap were 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 Streptococcus 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., 2012a). ZnO NPs also show marked inhibition of biofilm formation and haemolytic 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 opdT and type III repressor ptrA (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 NPs 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 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 minutes.

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).

7. ANTIMICROBIAL ACTIVITY OF HYBRID NANOCOMPOSITES

One of the most noticeable contributions of nanotechnology to oral hygiene and health are the nanoparticles based dental materials with improved antimicrobial properties such as composite resins adhesive systems, nanoparticles based polymers, nanofillers, nanocomposites and endodontic materials (Cheng et al., 2012; Dwivedi et al., 2013; Hule and Pochan, 2007). As the addition of antimicrobial agents to dental materials may adversely affect their desirable physico-chemical properties including hardness and mechanical strength, development of such antimicrobial dental materials is not easy (Hanemann and Szabó, 2010). Fortunately, in some studies it was observed that dental materials developed with nanoparticles offer esthetic and strength advantages over conventional micro-filled and hybrid resin-based composite systems besides possessing strong antimicrobial activity and remineralizing capabilities (Saunders, 2009). These materials are also advantageous in terms of flexural strength, polishability, smoothness, and precision of shade characterization, and micro-hardness, as compared to conventional dental materials (Saunders, 2009; Cheng et al., 2012). However, before introducing nanohybrid and nanobased dental materials to routine practice, their success in long term clinical trials should be confirmed.

Kawahara et al. (2000), tested in-vitro the antimicrobial activity of silver zeolites against oral bacteria under anaerobic condition and concluded that silver zeolites may serve as useful vehicle to provide antibacterial activity to dental materials used even under anaerobic conditions such as deep in the periodontal pocket. Silver nanoparticles have been incorporated in a number of dental materials to improve their antimicrobial activity.

Silver containing MDPB (12-methacryloyloxy dodecyl pyridinium bromide) primer inhibited oral biofilms and secondary caries without compromising dentin bond strength or biocompatibility of MDPB (Zhang et al., 2013). Incorporation of Ag NP into hydroxyethylcellulose polymer gels did not adversely affect its homogeneity and fluidity but improved their antimicrobial activity significantly (Bruniera et al., 2014). Similarly, the addition of Ag NPs in dental resin improved their mechanical properties and also increased their antimicrobial activity against E. coli (Kassaee et al., 2008). 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). In another study, considerable antimicrobial activity of silver complexes of poly(amidoamine) (PAMAM) dendrimers and silver-PAMAM dendrimer nanocomposite solutions against S. aureus, P. aeruginosa, and E. coli (Balogh et al. 2001) was observed.

Tavassoli-Hojati et al., (2013) developed resin composites containing various concentrations of ZnO NPs (0-5 wt. %) and evaluated their physico-chemical 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).

The incorporation of ZnO NPs to chitosan based sealers reduced the adherence of Enterococcus faecalis to ZnO nanoparticulates-treated dentin significantly but did not alter the flow characteristics of sealer (Kishen et al., 2008). In addition to the in-vitro studies clinical trials of nanobased dental materials also endorse the use of these materials (Kudo et al. 1990).

8. TOXICITY OF ANTIMICROBIAL NANOPARTICLES

Indeed, there is a multitude of challenges in translating nanotechnology and nanoantibiotics, in particular, for clinical use. Nanoparticles exhibit greater toxicity on both cellular and systemic levels compared to their larger size because of greater

invasive nature of the nanosize (Katsnelson et al., 2015). Toxicity of these NPs need 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). Whether the exposure to the NPs is through inhalation, ingestion or topical also decides the degree of its toxicity. For example, it was demonstrated by Vandebriel and Jong (2012) that inhalation of ZnO NPs was much more detrimental than the dermal exposure, as evident from the in vivo studies on rats and guinea pigs. As the use of metal and metal oxide 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.

Although a number of in vitro studies on toxicity of metal and metal oxide NPs are available, but these studies could have been more meaningful for real risk assessment if sufficient details of the test conditions and of NPs have been provided to ensure their reproducibility.

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/LC50 value of ZnO NPs das 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). In another study the toxicity of 11 metal oxide nanoparticles on three mammalian cell lines were compared, six metal oxide NPs including, SiO2 and TiO_2 did not show toxic effects below 100 μg/mL. While, CuO and ZnO NPs, exhibit an average IC50 of 22.4 μg/mL and 57.3 μg/mL values for the three mammalian cell lines (Ivask et al., 2015). 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 TiO2 NPs (5nm) both show minimal toxicity below 100 μg/cm2. 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 H2O2/OH, decreased O2–, 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. Which clearly shows that ZnO is safer than Ag NPs. Vandebriel and De Jong (2012) in their review of mammalian toxicity of ZnO NPs has 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 TiO2 (140 nm) and concluded that this form of oxide exhibited low hazard potential in aquatic and mammalian species/cell lines following acute exposure. In an interesting study on the toxicity of Ag NPs, Böhmert et al., (2014) simulated the ingestion process by treating the Ag NPs with saliva and artificial gastric juices. They found that the particles were not very much affected by the treatment and hence their cytotoxic potential was also not very much affected.

As the use of NPs in oral hygiene is proposed epithelial cells in oral surfaces and alimentary canal will be exposed to these NPs. However, this exposure can also be minimized by careful mouth wash and by avoiding any accidental ingestions. Surprisingly, not enough reports on in vivo toxicity of these NPs are available. It has been shown that only 10-20% of ingested silver metal is absorbed in the alimentary canal mainly by duodenum and small intestine (Boosalis et al., 1987). In another in vivo study on terrestrial isopods Valant et al. (2012) found that ingestion of TiO_2 nanoparticles exhibit toxic effects only at a high concentration of 1000-2000 μg g-1 of feed.

9. DOSE OPTIMIZATION

Dose optimization is crucial for successful clinical translation as well as minimizing toxicity concern of nanoparticles. This includes doses that will be used in oral hygiene products and those that will be incorporated in dental materials to improve their antimicrobial activity. Many studies have reported the toxicity of nanomaterials to different cell lines and in vivo at unrealistically high doses that cannot be realistic in case of human exposure. It has been pointed out by Wang et al. (2008) that indicated intranasal instillation of 7.5 mg of nanoscale TiO_2 caused oxidative stress and brain inflammation in mice. However, these doses will correspond to intranasal instillation of 17.5 g TiO_2 NPs in human beings which are impossible. Therefore, for toxicity studies proper and realistic doses should be taken into consideration.

10. CONCLUSION

Recent developments in nanotechnology and the discovery of new nanomaterials in near future will have great impact on dentistry and oral health care. An increasing number of studies are demonstrating the antimicrobial property and the biocompatibility of these nanomaterials and their possible use in different areas of dentistry.

Incorporation of these nanomaterials to various dental materials remarkably improve their desired physico-chemical properties, such as hardness, toughness, high strength and wear resistance. Hence, their use in oral hygiene products, in composites, sealers, injectable biomaterials and dental restorative materials is envisaged. Many dental materials have already been developed through nanotechnology and are available for clinical application; particularly nanobased cements and resins.

However, for use in dentistry nanomaterials should be chosen carefully and should be tested rigorously in vivo, or at least in conditions mimicking the oral cavity such as buffer capacity of saliva, pH and mucosal contact. Moreover, the results obtained

with a nanomaterial should be reproducible to assure the quality. Their properties such as solubility, stability, and possible release of ions through dissolution should also be determined in addition to their shape and size. For successful clinical translation, these nanomaterials should also be cost effective, to compete with other low cost options already available in the market and should also strictly follow safety regulations. Long-term clinical studies are required for careful and practical toxicology evaluation of nanomaterials. As the toxicology of these nanomaterials are mostly tested in vitro more clinical studies are required to better understand their mechanisms of action. Lastly, dose optimization of such materials is also crucial as it is also well known that some nanomaterials exhibit the toxicity if used beyond permissible limits. To conclude, although a lot of work has been done on dental materials but there is a clear need to address many important questions for their successful introduction in to public domain.

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6. ACKNOWLEDGEMENTS

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7. CONFLICTS OF INTEREST

The authors declare no conflict of interest.