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Metal Based Nanoparticles, a Novel Approach to Control Biofilm: Review

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A B S T R A C T

Biofilm is a large collection of colonies containing sessile bacteria which are adhered onto the surface and embedded in the self produced biopolymers such as Extracellular Polymeric Substances (EPS). The surface may be present either in soil or aquatic system as well as in any medical devices or living tissue. These polymeric substances form a cohesive network that interconnects and immobilizes the biofilm cells helping them in mechanical stability. Production of biofilm ultimately helps the bacteria to survive the hostile environment such as different phagocytic cells encountered in living tissue and different harsh conditions like heat, cold, shear forces, desiccation that the inert surface is exposed to. Biofilms are often difficult to treat because of their inherent resistance to both host defens system and antimicrobial agents that ultimately results in persistent infection. Therefore, now a days different nano particles are targeted against biofilms by analyzing and manipulating various atoms using nano technology. This review aims to summerize the current novel solutions to fight against the multi-drug related problems using metal based nanoparticles to target the biofilms.

Introduction

Biofilm can be defined as a microbial community formed by number of sessile cells that are reversely attached first to a substratum and then on the interface to each other, embedded in extracellular polymeric substance (EPS) produced by the microorganism themselves (Davies, 1998; Decho, 2010). They exhibit different phenotypes from the wild ones with respect to their growth as well as few gene transcriptions (Donlan and Costerton, 2002). The planktonic bacteria, free floating microorganisms are the prerequisite for biofilm production (Chaudhari *et al.,* 2012). They are formed on solid or liquid surface as well as on the surface of the living tissue that can serve as their nutrient source (Huang *et al.,* 2009). The process of development of a biofilm is often influenced by the physico chemical properties of the underlying surface where they are attached (Hannig and Hannig, 2009). It may contain the bacteria of either of same or of different species that attach to inert or living surface (O'Toole *et al.,* 2000). Biofilm production is a common survival mechanism of pathogenic bacteria that ultimately leads to antibiotic resistance and reduce host immune clearance (Singh and Barbul 2008; O'Toole *et al.,* 2000). It has been reported that most of the persistent infectious diseases occur due to presence of biofilm (Hall-Stoodley 2004; Marsh *et al.,* 2000) and cause high cost involvement annually for treatment i.e. it almost exceeds 81 billion in United States (Flemmig *et al.,* 2000). For the irreversible attachment on the surface there are certain receptors either protruding through or attached to the cell membrane that can bind to the ligands on the surface with strong but non covalent bonds (Busscher *et al.,* 1987). Usually in the extracellular polymeric substance matrix many substances are present like extracellular proteins, phospholipid, teichoic acids, nucleic acids and exo polysaccharides that are produced by the microorganisms within the biofilm. However, there is also evidence of presence of silts, mineral salts, blood components and milk residues depending on the sites and the environmental condition under which the biofilms are produced (Costerton *et al.,* 1995). The occurrence of biofilm is mostly studied in dental plaque or dental carries like infections, medical device related infections, systic fibrosis and different water bodies like marine surfaces, natural water as well as the waste water processing systems and even on the soil particles (Costerton, 1999). The bacteria can produce various signalling molecules to communicate with other bacteria of either same or different species or sometimes even with the host in response to change in the environment through altering the gene expression which is often density dependent or growth stage dependent (Holban and Lazar, 2011). The switching of the microorganisms from growth phase to transient dormant phase makes them resistant to different antimicrobial agents as well as drugs (Keren *et al.,* 2004). In this review it is discussed about controlling biofilm formation by means of the novel approach nanotechnology which improves the poor penetration of antibiotics or any drug in the biofilm.

The trends in nanotechnology can be used as an alternative to traditional antibiotics for effective extermination of biofilms. The word "Nano" is a Greek word which means dwarf so the term nanotechnology is basically manipulation of atoms by atoms (Schleyer, 2000; Whitesides, 2001).

Different inorganic nanoparticles are gaining attention as new approach to combat the challenge against antibiotic resistance. The metal ions like gold, copper, silver, zinc, iron and titanium are adopted for various research works to see their antimicrobial activities (Ramasamy and Lee, 2016). Out of them Silver metal has shown to be superior in terms of antibacterial activity that acts on the sulfhydril groups of the bacterial wall (Loh *et al.,* 2009) but high dosage of silver particle has toxic effect on the skin cells (Kostenko *et al.,* 2010).

Silver nano particles

The interaction between the EPS and bacteria plays an important role in the susceptibility of biofilm to the Silver nanoparticles (Ag-NP). Sheng and Liu (2011) found that the viability of the biofilm was reduced after removing the loosely bound EPS from the biofilm but the original waste water biofilm was somewhat tolerant when same amount of the Ag-NP (200mg Ag/L) was added under similar condition. Secinti *et al.,* (2011) studied that the silver coating on the titanium implant can reduce formation of biofilm than the non coated implant. Fabrega (2011) found that Ag particles are effective against natural marine biofilm forming bacteria. Zhang *et al.,* (2012) found that quaternary ammonium dimethacrylate along with silver nanoparticle has potentiated activity against the dental plaque microcosm biofilm than using the agent alone, by reducing the biofilm viability when added into the adhesives. Fei *et al.,* (2013) used *Bombyx mori* silk fibroin as biotemplate to produce silver nanoparticle as it acts as reducing as well as stabilizing agent for silver under incandescent or sunlight at room temperature and showed to be effective against MRSA and subsequently prevented biofilm production by the same bacteria. Di Giulio *et al.,* (2013) studied the antibacterial activity of nanocomposite system based on lactose substituted chitosen and the silver particles on saliva derived biofilms caused by *Streptococcus mitis, S. mutans, S. oralis* and found to be promising antibiofilm agent. Loo *et al.,* (2014) formed stable hydrogel incorporating Ag nanoparticles with polyvinyl alcohol where silver particles interacts with the OH group and acts as non toxic agent on human bronchial epithelial cells was found to have stronger antibiofilm effect on *Pseudomonas aeruginosa*. Freire *et al.,* (2015) evaluated antibiofilm property of Ag-NP using parallel-flow cell and dichromatic fluorescent stain system against Streptococcus mutans in dental enamel. Goswami *et al.,* (2015) synthesized Ag-NPs using tea extract and showed to have antibiofilm activity on silicon tubes and polystyrene coverslips with dose dependency disrupting cell-cell adhesion. Hazer *et al.,* (2016) studied antibacterial effect of polymer based Ag-NP coated pedicle screws on MRSA in the surgical sites of rabbit spine and found to be effective in minimizing biofilm formation. Misba *et al.,* (2016) evaluated antibiofilm activity of photosensitizer toluidine blue conjugated with silver naoparticles on *Streptococcus mutans* by using light and were found to be more phototoxic due to generation of reactive oxygen species hence decreased biofilm formation. Dai *et al.,* (2017) synthesized Silver nanoparticles with different cationic membrane targeting ligands attached on it and studied that it could irreversively disrupt bacterial membrane and inhibit intracellular enzyme activity causing 80% eradication of drug resistant bacterial biofilm. Malaikozhundan *et al.,* (2017) synthesized Ag-NPs coated with *Solanum nigrum* fruit extract and found to be effective against biofilm formed by *Bacillus pumulis* and *Enterococcus faecalis*. Kumar *et al.,* (2017) biofabricated Silver nano colloid using aqueous Silver nitrate and latex of plants jatropha and *Lannea grandis* found to be effective against biofilm formed by *Candida albicans.* Green synthesized silver nanoparticles using Artemisia vulgaris (Kiran *et al.,* 2017) and marine endophytic fungus *Penicillium polonicum* (Neethu *et al.,* 2018) have been studied to have antibiofilm activity. Yu *et al.,* (2018) studied the silver nanoparticle decorated quercetin nanoparticles against biofilm formed by Escherichia coli during mastitis and was proved to have stronger antibiofilm activity. Gillett *et al.,* (2018) studied chemical composition of Ag-NP using X-ray photoelectron spectroscopy and 10.6 nm sized particle was tested against E. coli biofilm formation and found to be effective in slowing initial biofilm formation. Sambalova *et al.,* (2018) found that biopolymer fraction of biofilm organic matrix can reduce ionic silver (Ag) and thus stabilize the formed nano silver and found that the Ag particle biopolymer interface is dominated by carboxylate functional groups. Ogawa *et al.,* (2018) analysed the micro organism in the biofilm of Ag-NP dispersed silane based coated carbon that reduces biofilm formation in sea water using next generation sequencing. Hussain *et al.,* (2019) synthesized silver nanoparticles from *Pandanus odorifer* leaf extract and it showed MIC of about 4-16 ug/ml against both gram positive as well as gram negative bacteria. The exopolysccharide production was decreased by 61-79 and 84% in gram negative and gram positive organisms respectively. Vijayakumar *et al.,* (2019) evaluated antibacterial as well as antibiofilm activity of garlic clove based silver nanoparticle and found to have excellent antibiofilm activity on MRSA and *Pseudomonas aeruginosa* at 100ug/ml. Hamida *et al.,* (2020) synthesized Ag-NP using Desertifilum spp. and evaluated antibacterial activity against five different MRSA strains where it caused denaturation of bacterial cellular proteins and particle induced reactive oxygen species could decrease biofilm formation. Porter *et al.,* (2020) formulated silver nano particle incorporated into glass ionomer cements which was found to reduce bacterial colonization and thus have antibiofilm activity.

Gold nano particles

It has been studied that gold NPs alone have no or little bacteriocidal activity (Yu *et al.*, 2016). Since they are non cytotoxic so can be conjugated with different targeting molecules like active biomolecules, antibiotics to have antibiofilm activity against different multidrug resistant bacteria (W.Y. Chen *et al.*, 2010).

Khan *et al.,* (2012) studied photodynamic therapy of methylene blue conjugated gold particle and found to have type one phototoxicity in the biofilm caused by *Candida albicans*. Together with other metal oxide like iron oxide gold particles can eradicate biofilm on biomaterial implants (Sathyanarayanan *et al.*, 2013). Au-NP can be used to store and stable release of the nitric oxide in the biofilm dispersal activity (Duong *et al.*, 2014). Sherwani *et al.,* (2015) studied the methylene blue and toluidine blue sensitizers conjugated Au-NPs on *Candia albicans* biofilms produced in BALB/c mice. Boda *et al.,* (2015) tested antibacterial activity of ultra small gold nano particles with core diameters of 0.8 and 1.4 nm (Au0.8MS and Au1.4MS) against *S. aureus, S. epidermidis, E. coli* and *P. aeruginosa* which showed 5 log bacterial growth reduction in 5 hours and with two fold increase in the MIC dosage caused 80-90% reduction in the Staphylococcal biofilm viability. Meeker *et al.,* (2016) studied daptomycin loaded Au-NPs against Staphylococcal biofilm. Ahmed *et al.,* (2016) studied about the activity of Au-NP conjugated to chlorhexidine and found to eradicate the pre formed biofilm formed by *Klebsiella pneumoniae*. Gold particles conjugated with chitosan-streptomycin is specially useful against biofilms formed by gram negative organisms (Mu *et al.*, 2016). Hu *et al.,* (2017) synthesized surface adaptive Au-NPs by modifying the surface with pH responsive monolayers targeting acidic environment of the biofilm and found to have photothermal ablation activity on biofilm formed by MRSA. Ahiwale *et al.,* (2017) synthesized gold particles using bacteriophage and found that 0.2mM of compost could cause 80% biofilm reduction. The heat dissipated by Gold nano particles due to surface plasmon resonance upon optical irradiation is a new approach to kill bacteria in biofilm (Pihl *et al.*, 2017). Gold stabilized antibiofilm agent cinnamaldehyde can be used as nano construct as singly the compound is unstable (Ramasamy *et al.*, 2017). Lu *et al.,* (2018) evaluated that the imidazole capped chitosan gold particles had enhanced antibacterial effect to prevent biofilm formation. Alteriis *et al.,* (2018) studied peptide indolicidin coated Au-NPs and their application against Candida biofilm. Green synthesis of gold particles from phyto compound baicalein (Rajkumari *et al.*, 2017), hordeinine as reducing and capping agent (Rajkumari *et al.,* 2017), Cannabis sativa (Singh *et al.,* 2018), fruit juice Citrus macroptera as reducing and stabilizing agent (Majumar *et al.,* 2019) were found to have antibiofilm effect. There are other gold particles which are obtained from hydrogen-producing hyperthermophilic bacterial strain

Caldicellulosiruptor changbaiensis (Bing *et al.,* 2018), rhizome extracts of *Rhodiola rosea* (Singh *et al.,* 2018) and marine plant *P. tetrastromatica* (Salam *et al.,* 2020) are known to have antibiofilm activity. Zhang *et al.,* (2020) coated the dental aligner with 4,6-di amino-2-pyrimidine thiol modified Au-NPs and proved to have the antibiofilm activity against *Porphyromonus gingivalis*. Bhatia *et al.,* (2020) used Silver-Gold hybrid nonoconstructs against intracellular infection as well as biofilm production.

Copper nanoparticles

Copper nano particles are seen to have narrow spectrum of antibacterial activity (P.S. Murthy *et al.,* 2011). Their antibacterial activities are less potential as compared to other metals like silver or iron particles hence high concentration is required for effective control (Ren *et al.,* 2009; Esteban *et al.,* 2009).

Akar *et al.,* (2013) tested the antifouling performance of copper by using modified polyetherosulfone filtration membrane dispersed with Copper and selenium particles. Ghasemian *et al.,* (2015) found CuO-NPs to be effective against Listeria monocytogeges biofilm with MIC 16mg/L and 67% hydrophobicity decreasing attachment and colonization. Lewis Oscar *et al.,* (2015) studied CuO-NPs and found to be effective against *P. aeruginosa* with MIC 100ng/ml and 94% reduction in biofilm. Wonoputri *et al.,* (2015) studied about the effectiveness of copper complex-nitrite-ascorbic acid system for biofilm control causing increase level of nitric oxide on addition of copper particles. Essa *et al.,* (2016) demonstrated the antibiofilm activity of biological Cu-NPs treated with headspace gases generated by E. coli. Miao *et al.,* (2016) found that CuO-NPs (50mg/L) could result in deeper penetration depth of oxygen in biofilm. Suresh *et al.,* (2016) studied effective antibiofilm activity of CuNPs using Hydrazine hydrate as reducing agent and *Gum kondagogu* extract as stabilizing agent. Ashajyothi *et al.,* (2016) studied symbiotic antibiofilm effect of copper and zinc oxide nano particles with minimum inhibitory concentration of about 2-128ug/mL. Miao *et al.,* (2017) studied the antibiofilm activity of CuO nanoparticles against waste water biofilm. Pant *et al.,* (2017) fabrigated a polymer composite containing S-nitroso-N-acetylpenicillamine with addition of Cu-NPs in carbosil to increase nitric oxide release. Woźniak *et a.,* (2017) synthesized Cu-NPs from peppermint extract using green reduction method and studied the synergistic effect of nanoconstruct loaded rifampicin in biofilm matrix disintegration. Covarrubias *et al.,* (2018) found that copper-chitosan NPs are effective against cariogeneic *Streptococcus mutans* in dental plaque biofims. Pugazhendhi *et al.,* (2018) analysed the photolytic activity of synthesized iron doped CuO and found to have antibiofilm activity. Shanan *et al.,* (2018) synthesized Cu-NPs using *Eucalyptus camaldulensis and* CuNO3 and found to have excellent antibiofilm activity. Widyńska *et al.,* (2018) found silver nanoparticls to have stronger antibiofilm effect than the Cu-NPs in all concentration. Logpriya *et al.,* (2018) studied antibiofilm effect of chitosan-CuO nano particles and found to have 63% reduction at 100ug/ml against *P. aeruginosa*. Beltrán *et al.,* (2019) studied antibiofilm activity of ascorbic acid capped Cu-NPs where ascorbic acid acts as reductant and stabilizer. Arul *et al.,* (2019) studied about the synergistic combination of beta lactum antibiotic amoxyclav with CuO nanocubes against multi drug resistant bacterial biofilm. Ali *et al.,* (2019) studied terpenoid encapsulated Cu-NPs made of Eucalyptus globules leaf extract as reducing as well as stabilizing agent against biofilm through generating reactive oxygen species. Khodair *et al.,* (2019) studied the deletion effect of Cu-NPs on biofilm gene ElF against *Pseudomonas aeruginosa*. Singh *et al.,* (2019) studied about the downregulation of genes forming biofilms and membrane lipo protein synthesis damaging the cell surface of *Pseudomonas aeruginosa* by Cu-NPs. Wang *et al.,* (2019) studied glycol modified Cu9S8 NPs against implant related biofilm. Rasool *et al.,* (2019) studied the antibiofilm activity against fluconazole resistant *Candida albicans* biofilm. Lotha *et al.,* (2019) studied about Cu-NPs capped with biogenic phytochemicals like cassinopin as well as isoquercitin and their antibiofilm effect against MRSA. Elsayed *et al.,* (2020) found that disinfectant loaded Cu-NPs could be used for Staphylococcal biofilm eradication in poultry farm as well as in abattoirs. Punniyakotti *et al.,* (2020) sysnthesized Cu-NPs using *Cardiospermum halicacabum* leaf extract and it was found to be effective against biofilm formation by attaching the cell wall and inhibit the growth of bacteria.

Zinc nano particles

Zinc oxide nano particles are known to have better antibacterial properties with low cellular toxicities against organisms like *S. aureus*, *S. epidermidis*, *E. facecalis*, *B. subtilis* and *E. coli* as compared to other metal oxides (Jones *et al.,* 2008; J.H *et al.,* 2014). However, zinc resistance also has been reported for *P. aeruginosa* and *Proteus* (B.M *et al.,* 2013; Zhang *et al.,* 2013).

Seil *et al.,* (2011) incorporated ZnO-NPs into polyvinyl chloride, amolymeric biomaterial to study the antibiofilm activity. Dwivedi *et al.,* (2014) studied about characterization and biocidal potential of ZnO-NPs against reactive oxygen species mediated bacterial biofilm. Lee *et al.,* (2014) found that ZnO – NPs inhibit biofilm formation as well as the production of pyocyanin, pyochelin and haemolytic activity of *P. aerusinosa*. The potential anti bacterial as well as antibiofilm activities of ZnO-NPs have been reported in combination with other metals like Silver and zinc dopped mulite ceramic discs (Saleh *et al.,* 2011), sonochemical coating of CuO and ZnO NPs against *S. mutans* biofilm formed on tooth (Eshed *et al.,* 2012), Copper and ZnO-NPs (Khan *et al.,* 2013), of silver and zinc oxide (Patil *et al.,* 2014), Silver and ZnO-NPs against enterotoxic *E. coli* (Salem *et al.,* 2015), Magnesium doped ZnO-NPs (Iribarnegaray *et al.,* 2019); graphene oxide supported zinc-cobalt oxides with high catalytic activity (Yang *et al.,* 2019), microspheres silver-zinc oxide NPs with polyester surfaces (Fontecha *et al.,* 2020), ZnO and silver nanopartcles in biofilm of MRSA from burn wound infection (Shakerimoghaddam *et a.,* 2020). Green extract of ZnO-NPs have been reported from various sources like *Plectranthus amboinicus* against MRSA (Vijayakumar 2015), *Pseudomonus putida* culture (Jayabalan *et a.,* 2019), plant extracts olive leave tomato fruit and tomato fruit (Ogunyemi *et a.,* 2019), sea weed Ulva lactuca (Ishwarya *et al.,* 2018), Aloe vera extract (Ali *et al.,* 2016), aqueous leaf extract of Plectranthus barbatus (Vijayakumar *et al.,* 2017) with effective antibiofilm activities. Abdulkareem *et a.,* (2015) combined ZnO with hydroxyl appetite coated onto Ti discs and examined on saliva biofim. Xu *et al.,* (2016) compared the toxicity as well as the antibiofilm activity of ZnO-NPs and Zinc ions against Fluvial bioffilm. Cierech *et al.,* (2016) studied polymethylmethacrylate modified ZnO-NPs agains fungal biofilm *Candida albicans*. Jothiprakasam *et al.,* (2017) synthesized ZnO-NPs from egg albumin and studied the antibiofilm activity against fungus. However, Ouyang *et al.,* (2017) found that mature and intact biofilm is tolerant to ZnO-NPs, also the environmental constituent humic acid (HA) reduces the toxicity of ZnO towards biofilm by binding HA on Zn ion. Divya *et al.,* (2018) studied gelatin coated ZnO-NPs and found to have stronger antibiofilm activity against gram negative organisms than in gram positive organisms. Bhattacharyya *et al.,* (2018) found the anti biofilm activity of ZnO-NPs against S. pneumonia at 12ug/ml conc. Shakibaie *et al.,* (2019) studied the non oxidised form of Zn particle to see the anti biofilm activity using *Lavandula vera* extract with microwave irradiation. Mehta *et al.,* (2019) studied the therapeutic index as well as the effect of nanomicellar composites containing chitosan and ZnO against drug resistant bacterial biofilm. El-Shounya (2019) tested antibiofilm activity of Zinc peroxide nanoparticles against multi drug resistant *P. aeruginosa*. Kemung *et al.,* (2020) studied that ZnO-NP had effective anti biofilm activity at minimum 13.5ug/ml against MRSA showing concentration dependent anti-adherence. Saravanakumar *et al.,* (2020) found that ZnO-NPs conjugated to Beta-D-glucan of barley has anti biofilm effect against *Trichoderma*. Banerjee *et al.,* (2020) studied the antibiofilm activity of pancreatic doped ZnO-NPs against MRSA that are non toxic in bacteriocidal concentration. Hsueh Jing *et al.,* (2020) found excellent anti biofilm property in catheter coating with zinc loaded palygorskite nanocomposites. Janani *et al.,* (2020) evaluated photolytic and anti biofilm activity of Carbon stabilized ZnO-NPs and found to be 100% effective in biofilm inhibition at 10ug/L against S.aureus.

Iron nano particles

Iron oxide nano particles have some unique properties like superparamagnetism, their surface to volume ratio is also high with large biocompatibility as well as easily extractable property (Ranmadugala *et al.,* 2018). Subbiahdoss *et al.,* (2012) studied surface modified SPIONs (superparamagnetic iron oxide-NPs) with magnetically concentrated carboxyl graft and analysed anti biofilm activity against gentamicin resistant Staphylococcus. Anghel *et al.,* (2013) combined FeO-NPs with essential oil secreted by *Satureja hortensis* and studied antibiofilm effect against *Candida albicans*. Temperature has impact on biofilm formation, an increase in the temperature by 5°C or more can cause detachment of biofilm. Nguyen *et al.,* (2015) induced local hyperthermia by using poly oligo ethylene glycol methyl ethyl acrylate block poly mono acryloxyethylphosphate stabilized FeO-NPs and used against Pseudomonas biofilm to see the dispersion. Masadeh *et al.,* (2015) found that addition of combination of metal nano particles like cerium oxide and iron oxide can interfere with broad spectrum antibiotic like ciprofloxacin. Salman *et al.,* (2015) synthesized FeO-NPs from Lactobacillus fermentum and found to have 33.97% inhibition against S. aureus and 16.92% against E. coli. Salman *et a.,* (2015) studied antibiofilm activity of chlorhexidine carrier nanosystem, based on FeO magnetic NPs and found to have anibiofilm activity at 78ug/mL.

Herrling *et al.,* (2016) studied biosorption of silica coated FeO-NPs on heterophilic biofilm and it was found to be concentration dependent. Velusamy *et a.,* (2016) synthesized oleic acid coated FeO-NPs and found to be more effective against gram positive organisms than in gram negative organisms produced biofilms. Chitosan coated FeO-NPs are found to be effective at 500ug/mL concentration (Shi *et al.,* 2016). Geilich *et al.,* (2017) synthesized multicompartment nanocarrier containing both hydrophobic superparamagnetic iron oxide-NPs(SPIONs) and hydrophilic methicillin and found to be effective at 40ug/mL SPIONs and 20ug/mL antibiotic to eradiate biofilm formation. SPIONs against biofilm were also studied by (Taylor *et al.,* 2009, Taylor *et al.,* 2012, Durmus *et al.,* 2013, Javanbakht *et al.,* 2016, Al-Shabib *et al.,* 2018). Ramezani *et al.,* (2017) studied super paramagnetic FeO-NPs and found to be effective against strong biofilm produced by *P. aeruginosa* but also reported that FeO-NPs could enhance the biofilm formation in isolate-dependent manner by utilizing the FeO-NPs as iron source for their growth. Kanchana *et al.,* (2018) synthesized FeO-NPs from leaf extracts of *Simarauba glauca* and *Artocarpus artilis* and found to have more than 75% reduction in biofilm within 24 hours and another green extract from grey mangrove *Avicennia marina* (Ramalingam 2019) also found to have anti biofilm activity. There is evidence that Fe-NPs can induce biofilm formation in certain bacteria like *P. aeruginosa* (Borcherding *et a.,* 2014)but, synthetic nanoparticles like FeOOH-NPs have been proven to disrupt the biofilm architecture in concentration dependent manner in *Pseudomonas aeruginosa* with modulation of motility and virulence (Pham *et al.,* 2019).

Naha *et al.,* (2019) studied the activity of nanozyme (dextran coated-iron oxide nanoparticles) and found that they were specific in nature to target the acidic environment within the biofilm EPS. Patel *et al.,* (2019) synthesized solid lipid nano particles of silver sulfadiazine loaded with chitosan gel in combination with DNase-I that can degrade the DNAs present in the polymeric substance of biofilm ultimately helps in cell to cell adhesion and found to be effective against biofilm related wound healing complication. Arias *et al.,* (2020) used the chitosan coated FeO-NPs as carrier for miconazole antifungal drug and tested against Candida biofilm. Cusimano *et al.,* (2020) synthesized biogenic nanoparticles embedding the Ag-NPs with peculiar EPS and then added iron nano particles onto it and found to have anti biofilm activity due to release of silver ions in low Fe/Ag-EPS.

Titanium nano particles

TiO2 acts as photocatalyst and due to its inert nature it is considered to be non toxic metal oxide, hence safe for application. Different antimicrobial actions involved are oxidation of several internal enzymes, peroxidation of lipids thus generating reactive oxygen species in the micro organisms leading to cell death (Y.Ohko *et al.,* 2009).

Chorianopoulos *et al.,* (2011) studied photocatalytic activity of TiO2-NPs against Listeria monocytogenes and it showed fastest log reduction in the of bacterial biofilm i.e. 3 log CFU/cm² after 90 min. Dhandapani *et al.,* (2012) studied photo catalytic activity of TiO2-NPs by fixing it on glass slide and allowed to grow biofilm on it, found to have good photo catalyst by generating hydrogen peroxide on the interface. Khan *et al.,* (2013) studied photocatalytic activity of TiO2 decorated silver nanocomposite using electro chemically active biofilm. Schug *et al.,* (2014) studied the activity of TiO2-NPs with UV radiation on extracellular enzymes in the biofilm like β-glucosidase and L- leucin aminopeptidase and found to have reduced activity due to generation of ROS. Naik *et al.,* (2014) studied AgCl-TiO_2 NPs and found to have anti biofilm activity where titanium helps in controlled sustainable release of Ag particles. Jesline *et al.,* (2015) studied antibiofilm activity of TiO_2-NPs on MRSA and found to have maximum zone of inhibition of 14mm at 500ug/mL and minimum zone of inhibition of 11 and 12mm at 100ug/mL. Kamaraj *et al.,* (2015) evaluated anti biofilm effect of silver nano particle loaded TiO_2-NPs against *Pseudomonas* and *Bacillus* spp. Jomini *et al.,* (2015) tested the TiO2-NPs against both sessile as well as planktonic stage of bacterial community. Xiao *et al.,* (2016) evaluated the anti biofilm activity of TiO2-NPs against E. coli biofilm and found that it generated reactive oxygen species which further quenched auto inducer signals needed for quorum sensing phenomenon as well as suppressed expression of other genes like motA and rcsB required for biofilm formation. Khan *et al.,* (2016) used combination of ZnO and TiO_2 nano particles against Streptococcus mitis biofilm and found to be effective at very low conc i.e. IC value 37ug/mL for ZnO-NPs and 77 ug/mL for TiO_2-NPS by inducing oxidative stress.

Biswas *et al.,* (2017) studied TiO2-graphene quantum dot hybrid material in presence of UV light, which was found to have stronger photocatalytic activity in compared to TiO2 due to generation of reactive oxygen species. De Falco *et al.,* (2017) studied flame synthesized nanocomposite having TiO2 coating on aluminium substrate and found to have antibiofilm activity due to photo oxidation in presence of UV light. Lopes *et al.,* (2017) used Titanium oxide nano-particles on diamond like carbon film used on several medical implants to reduce the oxidation of the implant material as well as to reduce bacterial biofilm. Dias *et al.,* (2019) synthesized TiO2 and TiO2/Ag NPs using microwave assisted hydrothermal method against *Streptococcus mutans* biofilm formation on resin surface.

Rajkumari *et al.,* (2019) tested the antibiofilm activity of TiO_2-NPs produced by the leaf extract of *Aloe barbadensis* after exposing the metabolic activity of *Pseudomonas aeruginosa* with MIC of TiO_2-NPs. Noreen *et al.,* (2019) synthesized nano composite combining Ag/TiO_2/grapheme and found to be effective against Campylobacter biofilm reducing the motility, hydrophobicity and self aggregation of C. Jejuni. Hou *et al.,* (2019) tested the TiO_2-NPs on periphytic biofilm and found that the metabolic activity like production of protein within the EPS was increased but the extracellular enzyme activity of alkali phosphatase was decreased when exposed to 1-5mg/L TiO_2-NPs.

In conclusion it is discussed in this review that nano technology plays a key role in creating different novel approaches to prevent and control biofilm formation. Several researches are being carried out to create different bio active compounds using metal oxide nano particles which target one or multiple species of pathogens. These metal oxide nano particles are proved to have bacteriocidal properties with minimum or no toxicity and hence safe for use specially to combat the emergence of antibiotic as well as multidrug resistance related problems. These can be synthesized from different sources including green source like leaf extracts and used as coating or drug carrier. Although different metal nano particles have been developed as novel anti biofilm agents but people fail to adopt them because of high cost involvement therefore, scientists are looking for alternative approach available considering the cost issue. Moreover, in future the risk of possibility of emergence of resistance against these metal nano particles should be analyzed thoroughly and the interaction among the particles, interface and host also should be taken into consideration for effective control of biofilm against different infectious agents.

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