Chapter 12
Metal and Metal Oxide Nanoparticles in Photoinactivation of Pathogens

Irena Maliszewska and Katarzyna Popko

Abstract Bacterial infections pose serious health problem that has drawn public attention worldwide. Increased outbreak and infections of pathogenic strains, bacterial antibiotic resistance, emergence of new bacterial mutations, lack of suitable vaccine and nosocomial infections are global health hazard to human. Over the last few years, the increased attention of the researchers was directed to questions related to the biomedical use of different nanoparticles. Nanotechnology is a research hot spot in modern materials science. This technology can provide new applications that range from innovative fabric compounds, food processing and agricultural production to medicinal techniques.

This chapter summarizes the experimental results of the effect of metal (like silver, gold) and metal oxide nanoparticles (like zinc oxide or titanic oxide) and quantum dots on the microorganisms under light exposure. The following sections discuss the properties of gold nanoparticles in photothermal killing of various pathogens, the ability of the conjugates of magnetic and plasmon-resonance nanoparticles with dyes, porphyrins and phthalocyanines to kill microorganisms as well as photocatalytic properties of ZnO and TiO_2 in inactivation of microorganisms.

Keywords Metal nanoparticles • Oxide nanoparticles • Photodynamic inactivation • Microorganisms • Bactericide enhancement • Fungicide activity

12.1 Introduction

Since the discovery, antimicrobial medications have proved remarkably effective for the control of microbial infections. It was, however, found out that some pathogens very soon became resistant to many of the first-generation drugs. Research into

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M. Rai, R. Shegokar (eds.), *Metal Nanoparticles in Pharma*,
DOI 10.1007/978-3-319-63790-7_12

and development of new antimicrobial medicines, vaccines and diagnostic tools call for more innovation and investment.

Photodynamic inactivation (PDI) of microbes is one of the innovative and promising approaches that involves reactive oxygen species (ROS) to kill microorganisms by combining a non-toxic dye termed a photosensitizer (PS) with low-intensity visible light, which, in the presence of oxygen, produces a cytotoxic species (Hamblin and Hasan 2004; Kharkwal et al. 2011). Typically, type I reactions generate radical and radical anion species (e.g. $O_2^{\bullet -}$, $HO^{\bullet}$), while type II reactions produce reactive singlet oxygen ($^1O_2$). For the type II pathway, the impact of PDI is highly dependent on the oxygen content, which is the presence of oxygen. The mechanism of action of PDI is a multi-target damaging process without any specific photosensitizer–receptor interaction on the surface or inside microorganisms. The multiplicity of cellular targets in microorganisms should reduce the risk of selection of photo-mutant resistant strains, and this risk should be further minimized by the lack of mutagenic effects of PDI (Plaetzer et al. 2009). PDI turned out to be a particularly good technique for dental and dermatological applications, involving the light irradiation of a tissue containing microorganisms that were previously exposed to a photosensitizing dye.

It is well known that susceptibility of bacteria to PDI is related to the structures of cell envelopes. Gram-positive bacteria are more sensitive to photodynamic inactivation than Gram-negative cells. The outer membrane of Gram-negative bacteria contains lipopolysaccharides and facilitates non-vesicle-mediated transport through channels. The complex structure of the cell wall makes the Gram-negative bacteria poorly permeable for photosensitizers and generated reactive species.

This therapy has some limitations particularly associated with the delivery of PSs (Ricchelli 1995; Lu et al. 2008). Most of the photosensitizers are hydrophobic and tend to aggregate in aqueous solutions, resulting in a reduced singlet oxygen generation (Kuznetsova et al. 2003). Therefore, in order to overcome this limitation, there is a great interest in developing photosensitizers based on nanoparticles incorporating PSs. Various approaches to utilizing nanoparticles for PDI have been reported, mostly using nanoparticles as a photosensitizer carrier. Nanoscale materials have unique physicochemical properties including extremely small size, high surface-to-mass ratio, high reactivity and special interaction with biological systems. By loading PSs into nanoparticles through physical encapsulation, chemical adsorption or coupling, many aspects of PDI can be improved, such as light dosimetry, photosensitizer bleaching, production of singlet oxygen, pharmacokinetics and drug therapeutic index.

The aim of this chapter is to discuss the use of plasmonic, magnetic, semiconductor and metal oxide nanoparticles in photodynamic inactivation of microbes.

12.2 Gold Nanoparticle-Mediated Photo Killing of Microorganisms

Gold nanoparticles (AuNPs) are considered to be very interesting nanomaterials in the photodynamic inactivation (PDI) field. Features such as oxidation resistance, biocompatibility or physical and chemical stability make AuNPs attractive for

various antibacterial technologies. Their optical properties can be evaluated by nonlinear optic measurements (Z-scan) (Olesiak-Bańska et al. 2012). In addition, synthesis or separation of these agents is easy (Guo and Wang 2007; Huang et al. 2009; Gordel et al. 2014).

In 2006, Zharov et al. described a new method for selective laser killing of bacteria targeted with light-absorbing gold nanoparticles conjugated with specific antibodies. AuNP-mediated PDT causes physical damage to the bacterium by using a combination of pulsed laser energy and the nanoparticles attached to the bacterium. When nanoparticles are irradiated, they absorb energy, which is quickly transformed through nonradiative relaxation into heat and other accompanying effects. All these factors eventually lead to irreparable damage to the bacterium. Efficient killing of *S. aureus* (above 90%) was achieved with 40 nm gold nanoparticles conjugated to a secondary IgG that targeted the bacterium by the use of a primary protein A (Zharov et al. 2006).

Norman (2008) performed covalent linking AuNPs to primary antibodies. The selectivity obtained through that linking was essential to destroying pathogenic Gram-negative *Pseudomonas aeruginosa*. There was a 75% decrease in cell viability after exposure of nanorod-coated cells to NIR radiation, which corresponded to a significant increase in the number of dead or compromised cells. It was suggested that cell membrane damage following nanoparticle exposure to NIR radiation could be due to numerous factors, including a nanoparticle explosion, shock waves, bubble formation and thermal disintegration.

Research concerning AuNP-mediated PDI is done with lasers working in the red and/or infrared spectral ranges. Investigation focused on the use of Au nanorods conjugated with photosensitizers or antibiotics to ablate MRSA via simultaneous PACT and NIR photothermal radiation. Au nanorods and nanocages simultaneously served as photodynamic and photothermal agents to deactivate MRSA (Kuo et al. 2009; Meeker et al. 2016).

Huang et al. (2007) proved that vancomycin-bound gold nanoparticles are capable of selective binding onto the cell walls of pathogenic bacteria. A large portion (>99%) of bacteria targeted by the AuNPs was destroyed under illumination by NIR light within 5 min. Next the inhibition of pathogenic bacteria cell growth, including Gram-positive, Gram-negative and antibiotic-resistant bacteria, was investigated (Huang et al. 2009).

Jijie et al. (2016) noticed an additional modality of nanoparticle-based photoinactivation of *E. coli* in their research on gold nanorods. The authors explain that antibacterial activity under pulse laser light is linked to the generation of singlet oxygen with the capacity of reducing bacterial viability by 2.5 $\log_{10}$. As ROS are highly reactive, they are believed to penetrate about 200 nm into solution from the site of the generation.

A photothermal effect of gold nanoparticles was observed during the biogenic AuNP-enhanced, MB-induced phototoxic effect on *S. epidermidis*. Irradiation with He–Ne laser (632 nm) caused significant kills in the presence of the free AuNPs. After 5 and 10 min of laser irradiation, the viable count showed a reduction of 1.84 and 2.04 $\log_{10}$, respectively (Maliszewska et al. 2014).

12.3 Conjugates of Gold Nanoparticles

It is well known that various molecules could be successfully attached to the surface of the gold nanoparticles and achieve their affinity in binding to the target cell. Therefore, improvement of sensitivity or bacteria detection speed in PDI methods by AuNP enhancement can be obtained. The detailed mechanism responsible for such phenomena is yet to be discovered although in many research papers, different hypotheses are advanced. Some of them discuss the AuNP presence that changes the relative distribution of ROS agents or increases their production. It is possible that various mechanisms are apparently involved in killing the bacteria, including the local increase in the concentration of the photosensitizer through targeted delivery of nanoparticles, selective interaction with the cell wall of bacteria and the resonance heating of AuNPs under laser light irradiation (Bucharskaya et al. 2016).

Researchers from Wellman Center for Photomedicine from Massachusetts admit that there are a few main advantages of using PS-containing nanoparticles (Sharma et al. 2012). First of all, the ability of the target cell to pump the drug molecule back out is limited; therefore the possibility of drug resistance is reduced. Passive or active targeting via the charged surface of the nanoparticle improves treatment selectivity, and finally the nanoparticle matrix is non-immunogenic (Konan et al. 2003; Pagonis et al. 2010).

It has been proven that the effectiveness of light-activated antimicrobial agents was amended by AuNPs combined with methylene blue in polysiloxane polymers. A significant antimicrobial activity has been shown against methicillin-resistant *S. aureus* and *E. coli*. A 3.5 $\log_{10}$ reduction in the viable count was obtained, when these bacteria were exposed for 5 min to light from a low-power 660 nm laser (Perni et al. 2009).

Perni et al. (2010) investigated the antimicrobial activity of light-activated silicon containing methylene blue (MB) and AuNPs of different sizes. In their study, they found that only gold nanoparticles of 2 nm diameter enhanced the antibacterial activity of MB, while 20 nm AuNPs reduced the antibacterial activity of the dye.

Another research concerning *S. aureus* was performed by Naik et al. (2011). Polyurethane polymer sheets embedded with MB or toluidine blue (TBO) as photosensitizer and 2 nm AuNPs also exhibited a good antibacterial activity. The incorporation of gold nanoparticles with the dyes enhanced the observed kill from 2.8 to 3.8 $\log_{10}$ (MB) and from 4.3 to 4.8 $\log_{10}$ (TBO).

Maliszewska et al. (2014) presented another promising PDI technique. Monodispersed colloidal AuNPs were synthesized by a reduction in Au^{3+} in the presence of *Trichoderma koningii* cell-free filtrate. Afterwards, these biogenic gold nanoparticles with MB as a photosensitizer were used as successful enhancers of *S. epidermidis* lethal photosensitization. MB showed a significant 1.5 and 1.8 $\log_{10}$ unit reduction in *S. epidermidis* after 5 and 10 min of irradiation by Xe lamplight. The AuNP–MB mixture showed a reduction in CFU of 4.7 $\log_{10}$ after 5 min of irradiation, which is a 99.997% kill compared with a 1.5 $\log_{10}$ or 95.238% kill for MB of the same concentration.

Besides the antibacterial potential described above, the AuNP–PS conjugates (MB or TBO) and 540 nm irradiation inactivated *Candida albicans* fungal biofilm. The AuNP-enhanced photodynamic therapy of MB against recalcitrant pathogenic *C. albicans* and *S. aureus* biofilm was also described (Khan et al. 2012; Darabpour et al. 2017). The treated cells lost their wall integrity and increased gold nanoparticle–methylene blue (AuNP–MB) conjugate permeability. The conjugate penetrated into the cell, causing cell wall disintegration and fragmentation of the nucleus or degradation of nuclear DNA.

It was suggested that the AuNP conjugate based on PDT could be employed effectively for treatment of cutaneous *C. albicans* infections in model animals (Mohd et al. 2015).

In 2007, it was demonstrated that toluidine O–tiopronin–GNP (GNP–gold nanoparticles) agents were much more effective in bacteria killing, compared to the non-enhanced with NPs toluidine O. The conjugate was active under both 632 nm laser and white light that significantly exceeded that of TBO. At a 1 mM concentration and a 30-min white light exposure, the conjugate showed a reduction in CFU of 4.5 $\log_{10}$, while 0.5 $\log_{10}$ value was obtained for TBO at the same concentration. The cited authors connected these results with the increased extinction coefficient of the conjugate compared to free toluidine (Gil-Toma's et al. 2007).

Tuchina et al. (2011) showed a 5% enhancement of photodynamic and photothermal effects after a 30-min laser illumination (λ = 808 nm) of *S. aureus* treated with conjugates of indocyanine green and gold nanocages.

A reduction in size of the *E. coli* has recently been achieved through a combination of gold nanorods with indocyanine green (ICG) photosensitizer (PS) and pulsed laser light (810 nm) (Jijie et al. 2016).

The combination of near-infrared (NIR) photothermolysis and photodynamic therapy against different models of bacteria (*S. aureus*, *S. epidermidis*, both methicillin susceptible and resistant) was shown by Ratto et al. (2011).

The antibacterial effect of conjugates of gold nanoparticles with dyes and porphyrins against *S. aureus* is compared in Table 12.1. These results show the strong bactericidal effect of these conjugates, and it seems that gold nanocages AuNCg2–haematoporphyrins conjugate show the highest antistaphylococcal activity.

One of the latest studies reports on a successful photodynamic inactivation of planktonic and biofilm cells of *C. albicans* using Rose Bengal (RB) in combination with biogenic gold nanoparticles synthesized by the cell-free filtrate of *Penicillium funiculosum* BL1 strain. Spherical gold nanoparticles (24 ± 3 nm) were coated with proteins; a Xe lamp (80 mW) was used as a light source. Rose Bengal showed a significant 1.74 $\log_{10}$ and 2.19 $\log_{10}$ unit reduction in planktonic cells of *C. albicans* after 20 and 30 min of irradiation. The RB + AuNP mixture showed a reduction in CFU of 4.7 $\log_{10}$ and 4.89 $\log_{10}$, after the same amount of time, that is, a 99.91% and 99.99% kill compared with that of 98.21% and 99.37% for RB of the same concentration. The authors presume that the enhanced phototoxic effect of RB by the biogenic gold nanoparticles may involve improvement of RB accumulation in *C. albicans* cells and a change of the main oxidative mechanism of Rose Bengal from type II to type I photosensitization (Maliszewska et al. 2017).

Table 12.1 Antibacterial effect of conjugates of gold nanoparticles against *S. aureus* 209P

Abbr.	Nanoparticle Shape	Photosensitizier (PS)	Average size, nm	Type of radiation	Maximal inhibition of *S.aureus* 209 P after 30 min-light exposure; CFU, % (Reference)
AuNRd1	Nanorods	ICG	30 × 10	808 nm, 50 mW/cm^2	65 (Ratto et al. 2011)
AuNS	Nanoshells	ICG	140	805 nm, 46 mW/cm^2	55 (Tuchina et al. 2011)
AuNCg	Nanocages	ICG	53	808 nm, 60 mW/cm^2	64 (Tuchina et al. 2011)
AuNR2	Nanorods	HP	50 × 10	808 nm, 100 mW/cm^2	90 (Khlebtsov et al. 2013)
AuNCg2	Nanocages	HP	50	625 nm, 100 mW/cm^2	97 (Khlebtsov et al. 2013)
AuNCl	Nanoclusters	PhS	1.8 (25 Au atoms)	660 nm, 50 mW/cm^2	90 (Khlebtsov et al. 2015)

ICG indocyanine green, *HP* hematoporphyrins, *PhS* Photosens™

Intensively studied, a new type of fluorophores–nanoclusters (NCs) is also emerging in PDT. Highly fluorescent bovine serum albumin (BSA)-directed Au–BSA NCs combined with human antistaphylococcal immunoglobulin (antiSAIgG) and Photosens™ used as photosensitizer created an effective complex. Khlebtsov et al. (2015) and Khlebtsov and Dykman (2017) demonstrated that photodynamic treatment of methicillin-sensitive and methicillin-resistant *S. aureus* with Au–BSA–antiSAIgG–PS complexes and 660 nm light irradiation significantly inactivates both types of bacteria.

12.4 Quantum Dots as Photosensitizer

One of the main predictions regarding quantum dots (QDs) as photosensitizer was made by Bakalova et al. in (2004), who suggested the possibility for energy transfer between quantum dot particles and cell molecules (as triplet oxygen, reducing equivalents, pigments). Potentially, this particular feature could induce the generation of reactive oxygen species, which may provoke apoptosis in cells (Bakalova et al. 2004). Quantum dots are known to be highly selective energy donors (Fisher et al. 2004). They are species with well-defined size, shape and composition and can be synthesized by relatively simple and inexpensive methods. QDs have been shown to be non-toxic in the absence of light, but have the potential to be cytotoxic under irradiation. Notwithstanding, it is their tunable optical properties and surface chemistries that are the biggest advantage of quantum dots over molecular photosensitizers (Samia et al. 2009).

In order to improve antibacterial activity, Narband et al. (2008) used CdSe/ZnS quantum dots with toluidine blue O as a photosensitizer. The authors came to the conclusion that mixtures of QD and TBO enhance bacterial kills in solution upon irradiation with white light, but only at low QD concentrations.

According to the latest research by Chong et al. (2016), graphene quantum dots (GQDs) are a promising novelty for PDI application. It was revealed that GQDs can perform both anti- and pro-oxidant activities depending upon light exposure, which will be useful in guiding the safe application and development of GQDs in terms of their antibacterial properties. Upon exposure to blue light, graphene quantum dots accelerate the oxidation of non-enzymic antioxidants and promote lipid peroxidation, contributing to its phototoxicity.

12.5 Conjugates of Magnetic and Plasmon-Resonance Nanoparticles with Porphyrins and Phthalocyanines

One of the most important aspects in photodynamic inactivation of microbes is the synthesis of functional cationic nanomagnet–porphyrin hybrids. Some researchers have investigated the possibility of using the PDT technique not only for clinical but also environmental application, more specifically for the inactivation of pathogenic microorganisms in water and wastewater (Jemli et al. 2002; Carvalho et al. 2007; Kuznetsova et al. 2007; Oliveira et al. 2009). Carvalho et al. (2010) for the first time used magnetic nanoparticles functionalized with neutral and cationic porphyrins as antimicrobial materials. Three cationic hybrids *6*, *8* and *9* (Fig. 12.1) were synthesized, and their photodynamic therapeutic capabilities were investigated for *E. coli*, *Enterococcus faecalis* and T4-like phages. These selected bacteria and the T4-like phages are commonly used as indicators of the presence of pathogenic microorganisms in wastewaters. The obtained results showed that these multi-charged nanomagnet–porphyrin hybrids are very stable in water and highly effective in the photoinactivation of bacteria and phages. Their remarkable antimicrobial activity, associated with their easy recovery, just by applying a magnetic field, makes these materials into novel photosensitizers for water or wastewater disinfection.

More recently, these researchers carried out the synthesis of new nanomagnet-porphyrin hybrids with a $CoFe_2O_4$ core and studied the recycling and reuse capability of this type of hybrids in water contaminated with bacteria. Bacterial inactivation was examined by monitoring the bioluminescence of Gram-negative *Aliivibrio fischeri* during photosensitization. It was shown that the synthesized cationic nanomagnet-porphyrin hybrids were highly efficient in bacterial photoinactivation and sustained several photoinactivation cycles (recycling and reuse) (Alves et al. 2014).

Haematoporphyrin derivative (HpD), a closely related mixture of oligomeric photosensitizers from blood, is the first-generation sensitizer for use in clinical PDT that engages in some activity against both bacteria and viruses. Their use in, for example, the disinfection of open wounds might, therefore, be problematic on the

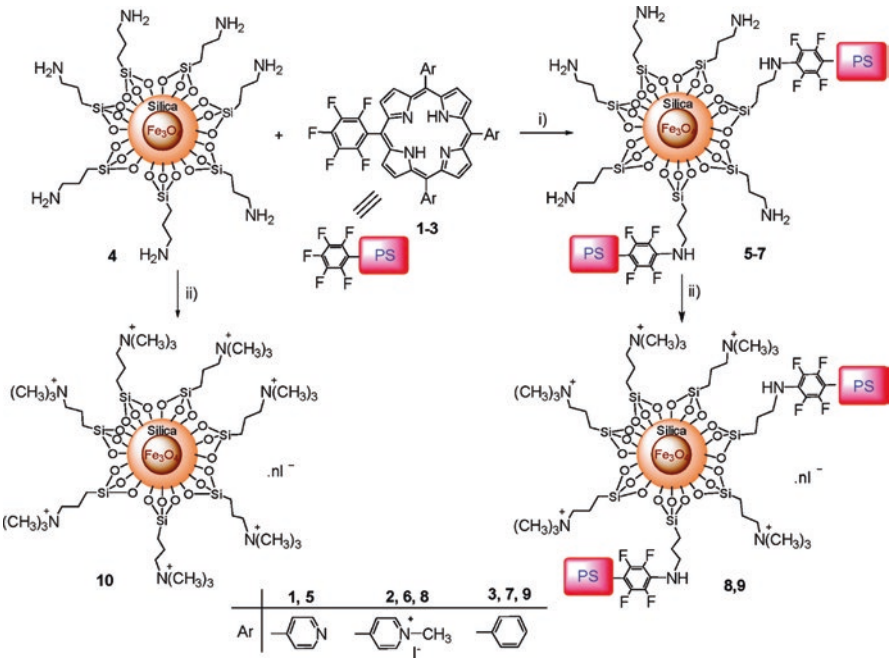

Fig. 12.1 Molecular structure of nanomagnet-porphyrin hybrids (Reproduced from Carvalho et al. 2010; American Chemical Society)

grounds of endogenous light absorption causing a decrease in photosensitizing efficiency, which is why some researchers adopted a new approach to this problem.

For example, Khlebtsov et al. (2013) prepared composite nanoparticles (gold nanorods) consisting of a plasmonic core and used a haematoporphyrin (HP)-doped silica shell. They observed an enhanced inactivation of *S. aureus* 209 P by nanocomposites in comparison with the reference solutions. After a 15-min irradiation with 405 nm light, the PDT efficiency of the composites was four to five times higher than that of free HP, and the percentage of the CFU was less than 3% in the case of incubation with NCs.

A further example of hybrids is presented by Hu et al. (2014), who described a successful synthesis of Ag@mSiO_2@photosensitizer hybrids. Ag@mSiO_2@HPIX (HPIX, haematoporphyrin IX dihydrochloride) was tested against *S. epidermidis* (ATCC 35984). The bacterial culture was mixed with the Ag@mSiO_2@HPIX hybrid and immediately irradiated with white light. The Ag@mSiO_2@HPIX hybrid displayed an enhancement in bacterial killing efficacy of 5 log when the concentration of the adsorbed HPIX was 2 mM. These authors also examined the antibacterial effect of the Ag@mSiO_2@HPIX hybrids against Gram-negative *E. coli* (ATCC 35218) and *A. baumannii* (ATCC 19606). For *E. coli*, the hybrid with an adsorbed HPIX concentration of 1 mM and under a fluence of 400 J cm^{-2} resulted in the complete eradication of the bacterium. In the case of *A. baumannii*, the same hybrid can completely eradicate the bacterium under a fluence of 200 J cm^{-2}. Thus it is clear

that the Ag@mSiO$_2$@photosensitizer hybrids display a synergistic effect in killing both Gram-positive and Gram-negative bacteria. The highly improved PDI efficiency of the Ag@mSiO$_2$@ photosensitizer hybrids was explained by (1) adsorption of photosensitizers in the mesopores of the silica matrix resulting in a very high local concentration, (2) the surface plasmon-photosensitizer coupling that enhances the singlet oxygen production efficiency and (3) the locally generated singlet oxygen that may reach a higher concentration than when free photosensitizers act individually, causing more damage to the bacteria.

Another study (Ding et al. 2016) showed the synthesis of silver nanoparticles (AgNPS) stabilized by poly(N-isopropylacrylamide-block-styrene; BCP) and used this material to entrap hydrophobic photosensitizing molecules (haematoporphyrin; HP). Synthesized AgNP@BCP@HP demonstrated a very high efficacy in the photoinactivation of *Staphylococcus epidermidis* (ATCC 35984) and *Escherichia coli* (ATCC 35218) under white light illumination. The enhancement of PDI efficacy was defined as $\log_{10}$ (enhancement killing) = $\log_{10}$ (AgNP@BCP@HP killing) − $\log_{10}$ (pristine HP killing) − $\log_{10}$ (AgNPs killing). A significant difference in the PDI efficacy against *S. epidermidis* among AgNP@BCP, pristine HP and AgNP@BCP@HP was thus clearly demonstrated. AgNP@BCP exhibited only a little bacterial killing, while pristine HP had moderate PDI efficiency under white light illumination. In contrast, AgNP@BCP@HP was characterized by higher PDI efficiencies than the sum of AgNP@BCP and HP. A similar effect was also observed in the PDI tests against the Gram-negative rods of *E. coli*, with a PDI efficacy enhancement of up to ~5 orders of magnitude. It should be noticed that AgNP@BCP@HP also photoinactivated bacteria under red/NIR illumination due to its broadened excitation profile. Again, AgNP@BCP@HP displayed a much higher PDI efficacy against both Gram-positive and Gram-negative bacteria than the summary efficacy of AgNP@BCP and HP. Moreover, it was also observed that in these red/NIR illumination experiments, the photoinactivation efficacy of AgNP@BCP@HP against *E. coli* appeared to be higher than that under white light illumination. This phenomenon was attributed to the longer illumination time (20 min) as compared to that under white light illumination (12 min), which may cause photothermal effect on the bacteria. This possibility was indirectly supported by the results of the control experiment involving only AgNP@BCP, where a longer illumination time also led to a higher killing efficacy.

Phthalocyanines have been studied extensively since the first synthesis in 1907 (Braun and Tcherniac 1907). The central cavity of phthalocyanines is known to be capable of accommodating 63 different elemental ions, including hydrogens (metal-free phthalocyanine, H_2-Pc). A phthalocyanine containing one or two metal ions is called a metal phthalocyanine (M-Pc) (Sakamoto and Ohno-Okumura 2009). Phthalocyanines and their derivatives, which have a similar structure to porphyrin, have been applied in important functional materials in many fields. Their useful properties are attributed to their efficient electron transfer abilities. Within the timeframe of the years 1930–1950, the full elucidation of the chemical structure of phthalocyanines was determined, and their X-ray spectra, absorption spectra oxidation and reduction, catalytic properties, magnetic properties, photoconductivity and many more physical properties were investigated. As a result of these studies, it was

concluded that phthalocyanines are highly coloured, planar 18 π-electron aromatic ring systems similar to porphyrins (De Diesbach and Von der Weid 1927).

In the last 20 years, phthalocyanine chemistry has been undergoing a renaissance because phthalocyanines and some of their derivatives exhibit singular and unconventional physical properties interesting for applications in materials science (Wöhrle et al. 2012). They are very stable and have strong absorption at short- and long-wavelength ends of the visible spectrum and are thus well suited for optical applications (Bonnett 1995; Kaliya et al. 1999; Loschenov et al. 2000; Makarov et al. 2007; Bohrer et al. 2009; Walter et al. 2010; Wang et al. 2012; Zhang et al. 2014). A great potential application is to use them as photosensitizers for photodynamic inactivation of pathogens (Minnock et al. 1996; Segalla et al. 2002; Caminos et al. 2008; Kussovski et al. 2009; Spesia et al. 2010; Di Palma et al. 2013; Zafar et al. 2016).

Great efforts of some researchers were focused on the efficiency improvement of photoinduced processes in phthalocyanines. In this connection, synthesis of hybrid exciton−plasmon systems based on metal nanostructures is considered to be one of the most promising solutions.

Nombona et al. (2012) described the photoinactivation activity of Zn phthalocyanine–polylysine conjugates in the presence of gold and silver nanoparticles against *S. aureus*. The obtained results showed that the antimicrobial efficacy of photosensitizers can be boosted by the presence of gold and more especially silver nanoparticles.

Masilela et al. (2013) reported on the axial coordination of zinc phthalocyanine and bis-(1,6-hexanedithiol) silicon phthalocyanine to silver and gold nanoparticles. An improvement in the photophysicochemical behaviour and antimicrobial activity was achieved in the presence of metal nanoparticles for both complexes. The bacterial inhibition was found to be best for the bis-(1,6-hexanedithiol) silicon phthalocyanine derivative in the presence of nanoparticles compared to the zinc phthalocyanine counterpart. The highest antimicrobial activity was achieved for both conjugates against *B. subtilis* compared to *S. aureus* both in the dark and under illumination with light.

Mthethwa and Nyokong (2015) reported on the effective complex of aluminium phthalocyanine in combination with gold nanorods (Fig. 12.2) for the photoinactivation of *Candida albicans* and *Escherichia coli*. The efficiency of this complex was evaluated by measuring the log reduction of the studied microorganisms after irradiation with visible light in the presence of photosensitizers. Aluminium phthalocyanine alone showed 1.78 $\log_{10}$ and 2.51 $\log_{10}$ reductions for *C. albicans* and *E. coli*, respectively. The conjugates showed higher photosensitization with 2.53 $\log_{10}$ and 3.71 $\log_{10}$ for *C. albicans* and *E. coli*, respectively.

12.6 Photocatalytic Properties of ZnO and TiO_2 for the Removal of Pathogens

The development of visible light-active materials for the removal of infectious pathogens has become very desirable. In general, wide band gap semiconductors such as ZnO and TiO_2 are recognized as efficient photocatalysts because of their

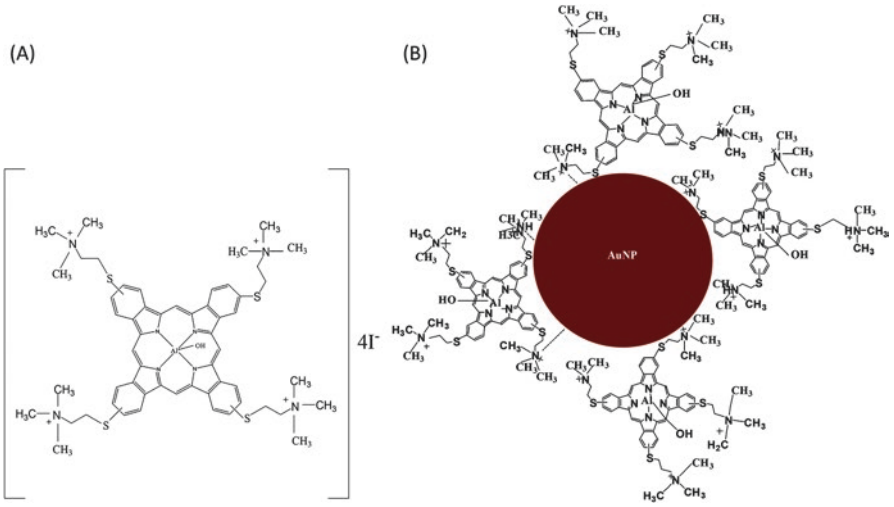

Fig. 12.2 (**a**) Molecular structure of the AlPc derivative (complex *1*). (**b**) Hypothetical structure based on the linking of complex *1* to gold nanoparticles (AuNPs) (Reproduced from Mthethwa and Nyokong 2015; Royal Society of Chemistry)

high redox potential of photocharge carriers. The mechanisms of the photocidal action of nanoparticles activated by light for microbial inactivation are different for various nanoparticles. It was found out that photocatalysis occurs in semiconductors such as ZnO and TiO_2.

12.6.1 Titanium Oxide Nanoparticles

One of the first reports concerning inactivation of bacteria and yeast under the presence of TiO_2 nanoparticles and the UV-A (360–400 nm) is the paper by Matsunaga et al. (1985). The authors reported that *Lactobacillus acidophilus*, *Saccharomyces cerevisiae* and *Escherichia coli* were killed photoelectrochemically with semiconductor powder (platinum-loaded titanium oxide, TiO_2/Pt; halide lamp irradiation for 60–120 min.). It was suggested that coenzyme A was photoelectrochemically oxidized, and the respiration of cells was inhibited, which caused death of the bacterial cells.

Later, it was shown that diffused solar light in the presence of TiO_2 resulted in the inactivation of various bacteria (Saito et al. 1992; Ireland et al. 1993; Wei et al. 1994; Kikuchi et al. 1997; Sunada et al. 1998; Shchukin et al. 2004; Robertson et al. 2005; Rao et al. 2006; Raulio et al. 2006; Kim et al. 2017) and viruses (Watts et al. 1995; Lee et al. 1997; 1998; Gerrity et al. 2008).

A large amount of experimental material related to TiO_2 nanoparticles' bactericidal effect under UV-A light illumination is known. The fact that the increased concentration of photocatalyst TiO_2 and UV-A light intensity results in a more rapid *Bacillus anthracis* killing has been considered non-direct evidence that the photocatalytic mechanism of their action occurs (Prasad et al. 2009; Sun et al. 2016).

An interesting observation was made by Bui et al. (2008). These authors suggested that the effective inactivation of bacteria is due to the contact between TiO_2 nanoparticles and the cellular wall. It was shown that bacterial cell and titanium dioxide adsorption significantly depends on the surface charge of TiO_2 nanoparticles and the bacterial wall. The surface charge of nanoparticles is determined by several factors, such as the isoelectric point, pH and electrolyte content.

Photocatalytic inactivation of microbial cells is schematically shown in Fig. 12.3. The absorption of light quantum with energy exceeding the width of a forbidden band in TiO_2 results in an electron and gap generation in conductivity and valence, respectively. Charges photogenerated in TiO_2 nanoparticles recombine or participate in reactions on the semiconductor–electrolyte division border, and electron reduces air oxygen to the superoxide of anion $O_2^{-\bullet}$ ($HO_2^{\bullet}$). The gap is responsible for the oxidation of hydroxide $H\bar{O}$ anion or the water molecule to the $HO^{\bullet}$ radical (Linsebigler et al. 1995). $HO^{\bullet}$ and $O_2^{-\bullet}$ radicals attack the organic molecules of a cell, which finally results in bacterial killing.

An interesting approach to this topic is modification of TiO_2 photocatalysts via doping by various atoms absorbing the light within the visible range. The effectiveness of bacterial inactivation in some cases increases under visible light and with TiO_2 doped by nitrogen and/or sulphur atoms (Bacsa et al. 2005; Liu et al. 2007; Vacaroiu et al. 2009; Zane et al. 2016) or Fe^{+3} (Rincon and Pulgarin 2007). There was observed an increased photocatalytic activity of TiO_2 under visible light (pure TiO_2 demonstrated rather little absorption under exposure to the visible light). Lately, researchers' interest in the field of the inhibition of pathogenic bacteria by TiO_2 has shifted to the study of TiO_2 nanoparticles containing such metal nanoparticles as Ag, Au, Pt, Pd, etc. The significant enhancement in bacterial killing was observed when the silver nanoparticles were inserted in TiO_2. The complete inactivation of *E. coli* was achieved in 1–2 min under exposure to UV-A light, whereas pure TiO_2 was shown to suppress bacterial viability in a suspension in about 30 min (Sokmen et al. 2001). The authors suggested that the enhanced bactericidal effect after silver application was due to increased effectiveness of organic cellular material oxidation in photocatalytic reactions. Changes in the kinetics of lipid oxidation and the malonic dialdehyde formation were reported while comparing the effects from the pure TiO_2 and Ag/TiO_2.

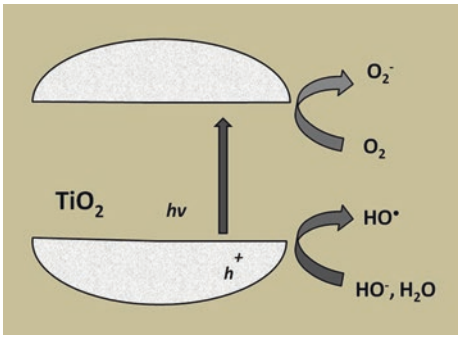

Fig. 12.3 Mechanism of the photocatalytic activity of TiO_2 nanoparticles (Adapted from Nadtochenko et al. 2010)

Some authors (Es-Souni et al. 2008) indicated that Ag/TiO_2 has reproducible bactericidal properties; however, the Ag/TiO_2 system might also possess bactericidal activity under dark conditions (without exposure to UV-A light). The said authors posit that the bactericidal effect of Ag/TiO_2 is due to the possibility of silver nanoparticles forming silver ions that are released into the solution.

The antimicrobial activity of nanostructural TiO_2 and TiO_2:In_2O_3 films and the effect of Ag or bimetallic Ag/Ni nanoparticles against *Pseudomonas fluorescens* and *Lactococcus lactis* were demonstrated by Skorb et al. (2008). The silver-modified TiO_2 film reveals the highest photo biocide efficiency, enhancing the bactericidal activity of UV light ca. 71-fold, which results from a radical improvement of microorganism adsorption and suppression of recombination of photo-produced charge carriers. The inactivation of Gram-negative *P. fluorescens* was higher than that of Gram-positive *L. lactis*. This difference was explained by the various structures of the cell wall in the Gram-negative and Gram-positive bacteria and the resistance of outer membranes to the reactive oxygen species generated by photocatalytic reactions.

Ag–TiO_2 was shown to possess a bactericidal activity against *E. coli* on the surface of hydroxyapatites (Reddy et al. 2007). A hydroxyapatite effectively adsorbs bacterial cells and Ag–TiO_2 inactivated them under the exposure to the visible light. Their combined action results in 100% bacterial death after 2 min. A study of this system using an electron microscopy technique revealed that photocatalyst nanoparticles adsorbed on the bacterial cellular surface obstructed cell nutrition. Moreover, it was shown that Ag–TiO_2 nanoparticles on hydroxyapatite inactivated bacteria in dark conditions.

Copper nanoparticles also demonstrate photocatalytic activity on the surface of TiO_2 nanoparticles (Sunada et al. 2003). Their study focused on the copper-resistant *E. coli* strain. Cu/TiO_2 has no influence on bacterial growth under dark conditions, but it completely inhibited bacterial growth under very weak UV light. It was shown that the decay curve of survival of the bacteria studied on the Cu/TiO_2 film under very weak UV light illumination consisted of two steps, similar to the survival change of normal *E. coli* on TiO_2 films under strong UV illumination. The first step was related to a partial decomposition of the outer membrane caused by photocatalytic oxidation, which further resulted in copper ions penetrating into the cytoplasmic membrane. The second stage was caused by a loss of cytoplasmic membrane integrity due to the copper ions, which brought about the loss of cell integrity. This two-step mechanism explains why the Cu/TiO_2 film system shows an effective bactericidal activity even under very weak UV light illumination.

Palladium nanoparticles were dispersed on two metal oxide substrates TiO_2 and SnO_2 (Erkan et al. 2006). The antimicrobial effect was studied for *E. coli*, *S. aureus*, *Saccharomyces cerevisiae* and *Aspergillus niger*. The antimicrobial efficiencies against different microorganisms and fungal spores were found to decrease in the following order: *E. coli* > *S. aureus* > *S. cerevisiae* > *A. niger* spores for which complexity and strength of the cell walls increased in the same order.

12.6.2 Zinc Oxide Nanoparticles

ZnO is known as a functional, promising and versatile inorganic material with a broad range of applications. ZnO is currently listed as generally recognized as a safe (GRAS) material by the US Food and Drug Administration (21CFR182.8991). Research on ZnO as an antimicrobial agent started in the early 1950s. During the last 50 years, it has been shown that ZnO can be used for many antimicrobial applications (Sirelkhatim et al. 2015).

It has unique optical, chemical and electrical properties (Fan and Lu 2005). It is characterized by a direct wide band gap (~3.3 eV) in the near-UV spectrum, a high excitonic binding energy (60 meV) at room temperature (Wang 2004; Janotti and Van de Walle 2009) and a natural n-type electrical conductivity (Wellings et al. 2008). The wide band gap of ZnO has a significant effect on its properties, such as the electrical conductivity and optical absorption. It was proved that ZnO nanoparticles in aqueous solution under UV radiation produce ROS such as hydrogen peroxide (H_2O_2) and superoxide ions (O^{2-}) (Fiedot et al. 2017; Prado-Prone et al. 2017). These generated active species penetrate into cells and are able to inactivate microorganisms. A detailed reaction mechanism of phototoxic antimicrobial activity of ZnO nanoparticles was proposed by Seven et al. (2004) and Padmavathy and Vijayaraghavan (2011). Photocatalysis was described as a photoinduced oxidation (Baruah et al. 2010).

ZnO is currently being examined as an antibacterial agent in both microscale and nanoscale formulations, and it is well known that this compound exhibits a particularly strong antimicrobial activity when particle size is reduced to a nanometre range. It was discovered that the improved antibacterial activity of ZnO nanoparticles compared to its microparticles was related to the surface area enhancement in the nanoparticles. Padmavathy and Vijayaraghavan (2008) investigated the antibacterial activity of ZnO nanoparticles with various particle sizes (12–2000 nm). These authors demonstrated that the bactericidal efficacy of ZnO nanoparticles increases with decreasing particle size. It was proposed that both the abrasiveness and the surface oxygen species of ZnO nanoparticles promote the biocidal properties of ZnO nanoparticles. More recently, the same authors (Padmavathy and Vijayaraghavan 2011) examined the antibacterial activity of ZnO nanoparticles with various particle sizes. The obtained results demonstrated that the bactericidal efficacy of ZnO nanoparticles also increased by decreasing particle size.

Prasanna and Vijayaraghavan (2015) conducted a systematic and complete antibacterial study on microparticle and nanoparticle of ZnO in both dark and light conditions. It was shown that micro ZnO in the dark revealed no activity against the bacteria studied. In light, ZnO nanoparticles exhibit a bactericidal activity remarkably higher than micro ZnO. Moreover, these studies have conclusively proved that reactive oxygen species (ROS) such as $^{\bullet}OH$, $^{\bullet}O_2^-$ and H_2O_2 are significantly produced from ZnO aqueous suspension even in the dark.

Zhou et al. (2008) showed the results of the antimicrobial activity of nanohydroxyapatite/zinc oxide upon UV exposure toward *E. coli* and S. *aureus*. Their findings also proved that the bactericidal activity can be achieved under UV illumination, ambient light or even in the dark.

Ann et al. (2014) studied the antibacterial activity against *E. coli* and *S. aureus* using ZnO of two forms (ZnO-rod and ZnO plate) which are exposed to UV-A illumination (390 nm). These authors found that UV-A illumination significantly influenced the interaction of both ZnO samples with the tested bacteria compared with unexposed ZnO by 13–21%. The antimicrobial activity depends on the shape of nanoparticles. Release of reactive oxygen species was proposed as an explanation of the mechanism of antimicrobial activity. The reactive oxygen species disrupted the DNA and protein synthesis of the bacterial cell, causing bacteriostatic effects toward *E. coli* and *S. aureus*.

Antimicrobial activity of photoactivated zinc oxide nanoparticles against human pathogens *Escherichia coli* O157:H7, *Listeria monocytogenes* ATCL3C 7644 and plant pathogen *Botrytis cinerea* was investigated by Kairyte et al. (2013). The obtained results suggested that ZnO nanoparticles in the presence of visible light exhibit a strong antibacterial and antifungal activity. Such properties could be used for the development of effective fungicides in agriculture or innovative physical antibacterial agents, so it is important in medicine and food microbial control.

The photocatalytic efficiency of modified ZnO was also evaluated for the inactivation of pathogens. For example, Guo et al. (2015) prepared a novel photocatalyst of Ta-doped ZnO nanoparticles. The antimicrobial study of the impact/influence of these nanoparticles on several bacteria *B. subtilis*, *S. aureus*, *E. coli* and *P. aeruginosa* was performed. The authors showed a particularly strong antimicrobial activity of Ta-doped ZnO nanoparticles under visible light irradiation.

Ta-doped ZnO nanoparticles exhibit an effective bactericidal efficacy due to the synergistic effect of enhanced surface bioactivity and increased electrostatic force (probably Ta^{5+} ions incorporated into ZnO).

ZnO nanoparticles with cobalt doping were synthesized by Oves et al. (2015), and antimicrobial properties were evaluated under sunlight deposition. The most effective bactericidal results were found for *E. coli* and *Vibrio cholerae*.

The F-doping was found to be effective against *S. aureus* (99.99% antibacterial activity) and *E. coli* (99.87% antibacterial activity) when irradiated with visible light. Reactive oxygen species production is one of the major factors that negatively impacts bacterial growth (Podporska-Carroll et al. 2017).

An interesting approach to this topic is the possibility to enhance the pathogen inactivation by combining ZnO and chlorophyllin. Chlorophyllin is a water-soluble food additive (E 140) known for its antimutagenic and anticarcinogenic properties and exhibiting a high antioxidant capacity (Kamat et al. 2000). The results obtained by Aponiene and Luksiene (2015) indicated that inactivation of *E. coli* by ZnO–chlorophyllin-based photosensitization is fairly effective.

In spite of many years of research, the exact mechanism of antimicrobial activity of ZnO particles has not been well understood. A number of mechanisms such as generation of hydrogen peroxide, accumulation of the particles on the bacteria surface, ROS generation on the surface of the particles, zinc ion release, membrane dysfunction, nanoparticle internalization and interruption of transmembrane electron transportation have been proposed as possible explanations.

12.7 Conclusions

The PDI is a truly promising approach to fight with highly pathogenic microorganisms. The variety of opportunities fuels constant development in this area of photochemotherapy. Different kinds of metallic nanoparticles like gold or zinc oxide nanoparticles can be involved in photosensitization process acting as a carrier that is conjugated with the selected dye. For instance, gold nanoparticles are often combined with such photosensitizers like MB, TBO or ICG, whereas plasmon-resonance and magnetic nanoparticles are conjugated with porphyrins and phthalocyanines. Crucial photocatalytic properties of quantum dots, ZnO, TiO_2 and gold nanoparticles are also intensively investigated in order to evaluate their antibacterial activity. Techniques presented here shed light on the potential environmental applications as well as on the improvement of already existing methods of PDI.

Acknowledgements This work was partially financed by a statutory activity subsidy from the Polish Ministry of Science and Higher Education (PMSHE) for the Faculty of Chemistry of Wrocław University of Science and Technology.

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