Photocatalytic nanoparticles
From membrane interactions to antimicrobial and antiviral effects
Parra-Ortiz, Elisa; Malmsten, Martin

Published in:
Advances in Colloid and Interface Science

DOI:
10.1016/j.cis.2021.102526

Publication date:
2022

Document version
Publisher’s PDF, also known as Version of record

Document license:
CC BY

Citation for published version (APA):
https://doi.org/10.1016/j.cis.2021.102526
Photocatalytic nanoparticles – From membrane interactions to antimicrobial and antiviral effects

Elisa Parra-Ortiz a, Martin Malmsten a,b,*

a Department of Pharmacy, University of Copenhagen, DK-2100 Copenhagen, Denmark
b Physical Chemistry 1, University of Lund, S-221 00 Lund, Sweden

ARTICLE INFO

Abstract

As a result of increasing resistance among pathogens against antibiotics and anti-viral therapeutics, nanomaterials are attracting current interest as antimicrobial agents. Such materials offer triggered functionalities to combat challenging infections, based on either direct membrane action, effects of released ions, thermal shock induced by either light or magnetic fields, or oxidative photocatalysis. In the present overview, we focus on photocatalytic antimicrobial effects, in which light exposure triggers generation of reactive oxygen species. These, in turn, cause oxidative damage to key components in bacteria and viruses, including lipid membranes, lipopolysaccharides, proteins, and DNA/RNA. While an increasing body of studies demonstrate that potent antimicrobial effects can be achieved by photocatalytic nanomaterials, understanding of the mechanistic foundation underlying such effects is still in its infancy. Addressing this, we here provide an overview of the current understanding of the interaction of photocatalytic nanomaterials with pathogen membranes and membrane components, and how this translates into antibacterial and antiviral effects.

1. Introduction

Infections cause millions of deaths each year and result in massive socioeconomic costs. Due to resistance development, bacterial infections are rapidly becoming non-responsive to conventional antibiotics [1–3]. The disease spectrum includes indications directly associated to the presence or action of a pathogen, but also diseases where microbes may result in an uncontrolled inflammatory state, eventually leading to sepsis. Sepsis remains the leading cause of death in intensive care units, with 20–40% overall mortality, and even higher for chronically ill and elderly patients [4]. Similarly, the SARS-Cov-2 pandemic has strikingly demonstrated our susceptibility to viral infections. Further emphasizing this is the occurrence of previous viral pandemics, including H2H2 and H3N3, HIV, Ebola, MERS, and SARS. This dramatically points to the need for finding new ways of combatting viral infection.

Nanomaterials may induce antimicrobial effects against both bacteria and viruses by several modes-of-action, including direct membrane destabilization, heat shock, or toxicity due to released ions. In addition, and the focus of the present overview, light exposure of photocatalytic nanomaterials may result in oxidative inactivation of pathogens [5–7]. In photocatalysis, light of energy exceeding the band gap of a semiconductor excites an electron (e–) from the valence band to the conduction band, creating a positively charged electron hole (h+) in the valence band in the process. Both the excited electron and the hole generated may subsequently react with water and dissolved oxygen to give reactive oxygen species (ROS) such as superoxide (O2 -) and...
Various nanoparticles display photocatalytic antimicrobial effects [12–14]. Among these, TiO$_2$ has attracted extensive interest. The photocatalysis of TiO$_2$, which depends on factors such as crystal structure, morphology, and crystallite size, is caused by ultraviolet (UV)-induced generation of ROS, particularly $\cdot$OH [9]. The photocatalytic activity of TiO$_2$ can also be enhanced by various approaches, including doping with metals, non-metals, and heterojunction coupling. Ultimately, these strategies aim to: (i) decrease the considerable band gap of TiO$_2$, thereby rendering it excitable not only by UV, but also by visible or even near infra-red (NIR) light, (ii) increase the number of free radicals formed, and (iii) decrease the e$^-$$\cdot$h$^+$ re-combination rate. Among such strategies, doping TiO$_2$ with transition metal ions is a powerful approach for extending photocatalytic effects into the visible light region, and to suppress charge recombination. Transition metal ions investigated for this include, e.g., iron, copper, tin, vanadium, and cobalt ions [9]. Coupling to noble metal nanoparticles, such as silver, gold, and platinum, is another approach for boosting TiO$_2$ photocatalysts, as is partial substitution of oxygen by carbon, nitrogen, or sulfur [9].

Quantum dots (QDs) constitute another type of photocatalytic nanomaterials of interest as antimicrobial agents. QDs are semiconductors with high quantum yield, resistance to photobleaching, and tunable photoluminescence. In addition to antimicrobial effects due to either direct membrane destabilization or ROS-mediated oxidation on illumination, QDs are interesting for theranostic applications, where the same system can be used for inducing antimicrobial effects and for monitoring the effects of the treatment [15]. Also metal-free photocatalysts, particularly carbon-based ones, are currently attracting considerable attention. Such materials include fullerenes, carbon nanotubes, graphene, carbon dots, and graphitic carbon nitride nanosheets (g-C$_3$N$_4$), which display facile synthesis and functionalization, high physicochemical stability, stable electron field emission, wide-band optical properties, and controllable band gap [16].

In the present overview, antimicrobial properties of these materials are outlined, together with the interaction of such materials with bacterial and viral membranes/envelopes, as well as with key components in these. In doing so, focus is placed on the interplay between photocatalytic effects and direct nanoparticle-target interactions. While illumination of various nanomaterials may result also in other types of antimicrobial effects, such as photothermal effects and photocatalytic acidification, the latter have been discussed in excellent previous reviews, and are not covered here [11].

2. Bacterial and viral membrane targets for photocatalytic nanomaterials

Apart from potent antimicrobial effects, selectivity is key for therapeutic applications of antimicrobial nanomaterial, so that bacteria and viruses are suppressed, while human cells are left unaffected. Here, differences in cell wall structure and composition between pathogens and human cells play an important role. The wall of Gram-negative bacteria consists of two lipid membranes with an intermediate peptidoglycan layer, the outer membrane rich in negatively charged lipopolysaccharides (LPS). The wall of Gram-positive bacteria, on the other hand, consists of a single lipid membrane surrounded by a thick peptidoglycan layer (Fig. 2A). Composition-wise, cholesterol is abundantly present (up to 45%) in human cell membranes, but absent in bacterial membranes [17]. In addition, while human cell membranes are generally dominated by zwitterionic phospholipids, (e.g., phosphatidylcholine (PC) and sphingomyelin (SM)), membranes of bacteria typically contain anionic phospholipids such as phosphatidylglycerol (PG), cardiolipin (CL), and lysisPG [18–21] (Fig. 2B).

Viruses are different from bacteria in their structure and cell wall composition. Thus, the virus surrounds its nucleic acid with a protein capsid, composed of a large but frequently specific number of one or more different capsid proteins (Fig. 3 (Right)). For example, cowpea chlorotic mottle viruses (CCMV) consist of 180 copies of a single 190-residue capsid protein arranged around roughly 3000 nucleotides. The capsid of retroviruses, on the other hand, contains one genomic RNA dimer, approximately 2000 molecules of nucleocapsid NC (a small protein rich in proline and basic residues), and 20–50 molecules of RT.
The capsid forms a strong but flexible shell, held together by hydrophobic and electrostatic interactions. For many viruses, the capsid is surrounded by a lipid membrane which is derived from one of the membranes of the host cells, such as the plasma, the endoplasmic reticulum, the Golgi complex, or the nuclear membrane, depending on the virus (Fig. 3 (Left)).

3. Interaction of photocatalytic nanoparticles with model membranes

In order to obtain mechanistic information on effects of photocatalytic nanoparticles with bacteria and viruses, studies with model membranes containing simplified structures formed by one or several
Advances in Colloid and Interface Science 299 (2022) 102526

key components of the pathogen membrane are helpful, as they allow careful compositional and structural variations to be investigated in well-defined systems, employing powerful analytical techniques. For bacteria, focus of such model system interactions has been placed on phospholipid membranes mimicking the membranes of bacteria [23]. In comparison, model studies using bacterial lipopolysaccharides (lipopolysaccharide (LPS) and lipoteichoic acid (LTA) from Gram-negative and Gram-positive bacteria, respectively) have been significantly less investigated, despite their importance for both infection and inflammation. Having said that, both LPS and LTA have been previously employed in mechanistic studies of antimicrobial peptides [24], and are likely to be able to provide valuable information also regarding antimicrobial nanomaterials. Similarly, model systems based on peptido-glycans have so far been rarely employed in model investigations of antibacterial effects. For viruses, investigations with capsid model systems are still in their infancy. However, since viruses acquire their lipid envelope from human cells, lipid models on eukaryotic cell membranes are applicable also for viruses. Nevertheless, there is a considerable untapped potential in studies of the interaction of nanoparticles with models of virus protein capsids. Since self-assembly of the latter is quite specific, and also depends on the interplay with nucleic acids, developing such model systems is, however, expected to require some efforts. Fortunately, considerable progress has been reached in the understanding of physicochemical mechanisms underlying capsid assembly [25], which can lay the ground for the development of such model systems.

3.1. TiO$_2$-based nanomaterials

Investigating lipid membrane interactions of TiO$_2$ nanoparticles, Pera et al. varied the charge density of both membrane and nanoparticles [26]. At low charge of the nanoparticles and/or the lipid bilayers, the absence of electrosstatic barrier caused fast binding, while binding was suppressed at high negative charge densities of particles and bilayers. In line with this, Zhao et al. found fast and irreversible adsorption of negatively charged TiO$_2$ to positively charged POPC/POEPC bilayers but limited binding to anionic POPC/POPS and POPC/POPG bilayers [27]. Furthermore, Sydor et al. found such TiO$_2$ nanoparticle binding to increase lipid order of (zwitterionic) POPC liposomes, whereas presence of cholesterol significantly suppressed effects of these nanoparticles on lipid order [28]. Correlating such direct nanoparticle-mediated membrane destabilization with antibacterial effects, Khat et al. investigated effects of TiO$_2$ nanoparticles on their effect on Escherichia coli (E. coli) and Staphylococcus aureus (S. aureus) membranes. TiO$_2$ nanoparticles of 8–10 nm in size were found to potently depolarize the membrane potential of Gram-negative E. coli bacteria, but not that of Gram-positive S. aureus. These effects correlated to antimicrobial activity results, and were also supported by membrane leakage for intracellular protein, DNA and K$^+$ [29].

Apart from direct particle-induced membrane destabilization, TiO$_2$ nanoparticles may destabilize membranes through lipid oxidation. Illustrating this, Arora et al. found mixed rutile/anatase TiO$_2$ to be effective against multi-drug resistant Pseudomonas aeruginosa (P. aeruginosa), but only on UV illumination (Fig. 4A) [30]. Addressing the origin of such effects, Erdem et al. reported on photogeneration of ROS, in turn triggering lipid oxidation (Fig. 4B) [31]. Analogously, TiO$_2$ nanoparticles have been reported to cause photocatalytic degradation of non-lipid bacterial components, such as LPS and peptidoglycan (Fig. 4C) [32]. Further illustrating photocatalytic lipid oxidation, Runa et al. investigated effect of TiO$_2$ nanoparticles on plasma membrane lipids. After TiO$_2$ nanoparticle exposure, plasma membrane lipids were found to be oxidized, based on malondialdehyde (MDA) quantification. This lipid oxidation was found to be suppressed by: (i) surface passivation of the TiO$_2$ nanoparticles, (ii) incubation with an antioxidant (Trolax), and (iii) presence of serum proteins (the latter adsorbing to the surface of the TiO$_2$ nanoparticles). On replacement of the corona proteins over time, however, this protective effects against TiO$_2$-induced lipid oxidation was reduced, and long-term oxidation levels similar to that found in the absence of protein corona [33]. Similarly, Maness et al. investigated antimicrobial effects of TiO$_2$ under illumination with near-UV light against E. coli, and found an exponential increase in MDA production on illumination, the kinetics of which paralleled bacterial killing [34].

Investigating the mechanism of oxidative destabilization of lipid membranes by anatase TiO$_2$ nanoparticles, Malekkhaht-Haffner et al. reported on effects of phospholipid charge and presence of cholesterol. While nanoparticle binding was found to have some destabilizing effect alone, particularly for membranes containing anionic POPG, UV illumination and ROS formation were found to dramatically accentuate membrane destabilization. Based on a battery of experimental techniques, it was demonstrated that anionic POPG renders the bilayers more susceptible to oxidative destabilization, whereas cholesterol provides a stabilizing effect. From this, it was suggested that less dense packing, or the occurrence of different free-radical processes, causes the larger sensitivity to degradation in bilayers containing anionic phospholipids (Fig. 5B,C) [35]. Furthermore, Parra-Ortiz et al. investigated UV-induced membrane destabilization by TiO$_2$ nanoparticles for POPC-based bilayers. Again, nanoparticle binding alone caused only minor bilayer destabilization. In contrast, UV illumination in the presence of TiO$_2$ nanoparticles triggered lipid oxidation and membrane destabilization through formation of ROS (notably $^\bullet$OH) (Fig. 5A). Furthermore, UV exposure in the presence of TiO$_2$ nanoparticles caused large-scale structural transformations especially at high ionic strength, including increased hydration, lipid removal, bilayer thinning, lateral phase separation, and aggregate solubilization [36]. In comparison, UV exposure

![Fig. 4](image-url)
of polyunsaturated POPC-based bilayers in presence of H$_2$O but in the absence of such nanoparticles was found by the same authors to result in quantitatively smaller effects [37].

Comparing effects of TiO$_2$ nanoparticles on lipid and non-lipid membrane components, Kiwi et al. investigated TiO$_2$-mediated photocatalytic oxidation of E. coli bacteria, as well as of LPS, phosphatidylethanolcholine (PE), and peptidoglycan (PGN). In doing so, photogenerated charge carriers were monitored by laser kinetic spectroscopy, while attenuated total reflectance–Fourier transform infrared spectroscopy (ATR-FTIR) spectroscopy allowed identification of photocatalytic oxidation products. From this, PGN was found to be the more resistant to photocatalytic degradation than LPS and PE [38]. Similarly, Liu et al. investigated antibacterial effects of exposure to TiO$_2$, UV light, and their combination for E. coli bacteria. In doing so, damage of the outer bacterial membrane was observed after treatment of TiO$_2$ or UV light. Whereas TiO$_2$ alone was found to be able to break down LPS in the outermost membrane of the E. coli cells, it was not able to destroy the peptidoglycan underneath. Furthermore, Ca$^{2+}$ and Mg$^{2+}$ were detected in supernatants of bacteria suspensions after photocatalysis, attributed to LPS damage [39]. Also demonstrating oxidative LPS degradation by TiO$_2$ nanoparticles under UV illumination, Sunada et al. investigated copper-deposited TiO$_2$ employing copper-resistant E. coli bacteria. While no decrease in bacteria survival rate was observed in darkness, UV illumination substantially reduced bacterial survival rate. The decay curve of bacterial survival was found to follow two steps, speculated to be related to: (i) partial photocatalytic destabilization of the outer membrane, and (ii) destabilization of the inner membrane by copper ions [40]. Furthermore, Wu et al. investigated the photocatalytic activity of TiO$_2$ nanoceramic coatings with oxygen vacancies, and found these to effectively degrade LPS under visible light. Despite this, in vitro cell toxicity against human MG63 cells was found to be comparable to that of untreated titanium substrates [41]. In another study, Kurz et al. investigated TiO$_2$-coated glass under UV-A exposure and found this to potently degrade LPS. The photooxidative degradation was found to be independent of the initial LPS concentration, following a zero-order reaction [32]. Since LPS induces potent inflammatory effects, TiO$_2$ nanoparticles may reach anti-inflammatory effects through oxidative LPS degradation (Fig. 4C). Also demonstrating this, Neascu et al. investigated the mechanisms by which TiO$_2$ nanotubes attenuate the inflammatory activity of macrophages. By using specific inhibitors, it was inferred that the anti-inflammatory effect of TiO$_2$ nanotubes occurred via suppression of the MAPK and NF-$\kappa$B pathways [42].

3.2. Metal nanoparticles

Ag, Au, and Cu nanoparticles cause potent antimicrobial effects, induced by direct membrane destabilization, binding to DNA and sulfhydryl groups in enzymes, as well as ROS-mediated oxidation [6,7]. Investigating direct membrane destabilization, Moghadam et al. reported positively charged Au nanoparticles (AuNPs) to be more efficient
in inducing leakage of zwitterionic DOPC liposomes than negatively charged nanoparticles [43]. Demonstrating the importance of nanoparticle surface properties for this, nanoparticles with identical surface functionality but different core were found to display similar effects on liposome leakage. From transition electron microscopy (TEM) results, leakage was inferred to be due to nanoparticle binding. Strikingly, a single nanoparticle was found to be sufficient to trigger complete leakage of a liposome.

Furthermore, Tatur et al. found that nanoparticles functionalized with cationic head groups bind to zwitterionic DSPC lipid bilayers and destabilize these [44]. In contrast, anionic AuNPs were found not to insert into the bilayers. Contrasting this, Bothun et al. investigated interactions of anionic carboxylated and cationic polyethyleneimine-modified AuNPs with spread lipid monolayers. The anionic nanoparticles were found to insert into DOPC/DOPG and DPPC/DPPG monolayers at low surface pressure, and to trigger monolayer condensation at higher initial surface pressure. The cationic nanoparticles, on the other hand, inserted only into DPPC/DPPG monolayers, despite more extensive binding [45]. Also demonstrating the complexity of this, Wang et al. investigated AuNP interactions with DMPG, DPPG, and DOPC, the latter having identical headgroup but different acyl chain composition. In doing so, liposome membrane fluidity was demonstrated to promote aggregation of membrane-bound particles, inferred to be due to faster diffusion within fluid bilayers, but also to local lipid gelation, followed by aggregation of gelled domains [46]. Similar effects were reported by Sugikawa et al. [47], while Montis et al. investigated the interaction of citrate-coated AuNPs with POPC membranes, and found AuNP clustering to be influenced by citrate-lipid ligand exchange [48].

Investigating the mechanism underlying lipid membrane destabilization by metal nanoparticles, Xing et al. investigated the interaction of AuNPs of different charge with DOPE/DOPG phospholipid membranes using small-angle X-ray scattering (SAXS) and confocal fluorescence microscopy. Although the model membranes carried a globally negative charge, local repulsive interactions between the AuNPs and the DOPE headgroups were found to induce a phase change, from lamellar to inverted hexagonal. Furthermore, transmembrane pores were observed in the inverted hexagonal structure, signaling on destruction of membrane barrier function [49]. Furthermore, Hu et al. employed vibrational spectroscopy for the investigation of membrane interactions of silver nanoparticles (AgNPs). It was found that AgNPs induce flip-flop of substrate supported DSPC lipid bilayers. Furthermore, nanoparticle aggregation after membrane binding was observed, but only at higher AgNP concentrations [50]. Moreover, Loliato et al. studied the interaction of cationic AuNPs with DSPC/DSPG membranes using neutron reflectometry (NR) experiments and molecular dynamics (MD) simulations to. From this, it was found that incorporation of AuNP into DSPC bilayers requires an energetic barrier to be overcome, e.g., by elevated temperature. In contrast, higher temperature was found to suppress AuNP incorporation into DSPC:DSPG (3:1), the latter also being associated with extraction of anionic DSPG lipids, demonstrating that both lipid charge and temperature are important for AuNP-membrane interactions. [51].

Addressing interactions between metal nanoparticles and non-lipid bacterial membrane components, Gomes da Silva et al. investigated polyvinylpyrrolidone-stabilized AuNPs incorporated in floating lipid monolayers in the absence and presence of bacterial lipopolysaccharides (LPS) or peptide glycan. In the absence of bacterial lipopolysaccharides (LPS or peptidoglycan), lipid adsorption to nanoparticles resulted in monolayer condensation. In contrast, monolayers containing LPS or peptidoglycan were found to expand upon AuNP incorporation [52]. Membrane interactions of metal nanoparticles may, however, also have other effects of relevance for their antimicrobial effect. Thus, Villameuva et al. reported on the interaction of hydrophobic oleic acid-coated AgNP with monolayers formed by phospholipids of different acyl chain composition. Incorporation of these nanoparticles into the monolayers was found to result not only in an expansion of the monolayer and component segregation, but also in release of Ag⁺ ions to reach bactericidal concentrations [53]. Similarly, in one of relatively few studies addressing mechanisms underlying the antimicrobial effects of metal nanoparticles alone under illumination (as opposed to such nanoparticles used in heterostructures), Rtimi et al. investigated killing of E. coli under visible light illumination for CuNP-polyester composites, and found that ROS play an important role in bacterial inactivation through oxidative degradation of LPS, but also that oxidation was paralleled by release of antimicrobial Cu²⁺ [54].

3.3. Quantum dots

Addressing the effects of nanoparticle surface properties, Xiao et al. investigated the interaction of carboxylated CdSe/ZnS QDs with cationic DOTAP bilayers. At pH 8.2, characterized by large QD-membrane charge contrast, bilayer disruption was pronounced, resulting in patched bilayers [55]. Close to the isoelectric point (IEP), on the other hand, individual QDs packed densely at the bilayer surfaces. Thus, at high pH, strongly negatively charged nanoparticles readily disrupted the positively charged bilayers. Close to the IEP, in contrast, weakly charged QDs bound to the bilayer, but did not destabilize it. Demonstrating the biological relevance of such effects, Wang et al. demonstrated transport of QDs across the plasma membranes of red blood cells, rich in zwitterionic lipids, as well as across model membranes with lipid compositions similar to those of red blood cell membranes. From such studies, QD incorporation was found to promote bilayer flexibility, but strikingly not to result in permanent defects [56].

In one of relatively few studies addressing mechanisms underlying the antimicrobial effects of QDs alone under illumination (as opposed to QDs used in heterostructures), Qu et al. investigated the photocatalytic efficiency of BiVO₄ against E. coli. It was found that the E. coli envelope was destabilized by BiVO₄ under illumination. Furthermore, ATR-FTIR results reported on reduced intensities for amide, phosphoric, and -COO⁻ groups, as well as for C-H bonds, suggesting light-induced degradation of LPS and peptidoglycan, as well as periplasm components [57].

3.4. Carbon-based nanomaterials

3.4.1. Fullerrenes

Fullerenes are carbon-based cage-like structures. This renders unmodified fullerenes colloidally unstable in aqueous solution, whereas hydroxylated fullerenol is readily dispersible. Addressing membrane interactions of such systems, Brousbois et al. investigated the effect of fullerenol on DPPC/cholesterol and DPPC/DPPG membranes by nuclear magnetic resonance spectroscopy (NMR) and Fourier-transform infrared spectroscopy (FTIR) [58]. Fullerol was found to display low affinity for zwitterionic DPPC/cholesterol bilayers but high affinity for anionic and cholesterol-free DPPC/DPPG membranes. Furthermore, while fullerenol nanoparticles were found to reside at the interface of DPPC/cholesterol bilayers, they located in the polar headgroup region of DPPG-containing membranes, thereby disturbing the acyl chain packing. Similarly addressing effects of membrane composition, Domingos Alves et al. employed MD simulations to investigate the interaction of C₆₀ fullerenne with DOPC bilayers containing various fractions of cholesterol. Extraction of DOPC and/or cholesterol molecules was compared with C₆₀ insertion into the lipid/cholesterol membranes in order to estimate the energy required for C₆₀ incorporation. In addition, experimental results obtained by electron spin resonance spectroscopy corroborated those obtained with MD simulations and indicated that cholesterol increased bilayer rigidity as well as the force required for C₆₀ insertion [59].

Investigating effects of nanoparticle aggregation on membrane destabilization, Hou et al. reported on the binding of fullerene aggregates (nC₆₀) and fullerol to eggPC bilayers [60]. Being more...
hydrophobic, membrane partition was found to be higher for nC₆₀ than for fullerol, although binding saturation was reached faster for fullerol than for nC₆₀. On a related theme, Ikeda et al. investigated fullerene exchange on addition of C₇₀/cyclodextrin complexes to giant unilamellar vesicles (GUVs) formed by POPC [61]. As a result of C₇₀ exchange, aggregates formed within the lipid membrane and caused GUV shrinkage. Clearly, aggregation therefore seems to be of importance for membrane interactions of fullerene derivatives.

3.4.2. Carbon nanotubes

Since carbon nanotubes (CNTs) are colloidal unstable in aqueous solution, surface modification is needed for biological applications of such ones. Even with surface modifications (anionic as well as cationic), however, CNTs frequently destabilize lipid membranes. For example, Corredo et al. investigated DOPC bilayers in the presence of anionic carboxyl-functionalized multi-wall CNTs (MWCNTs), and found the latter to readily destabilize the bilayers already at 1.6–12 ppm [62]. Furthermore, Cancino et al. studied cationic polyamidoamine-modified single-wall CNTs (SWCNTs) in combination with zwitterionic DPPC monolayers, and found that the CNTs were able to insert into these even at surface pressures up to ~30 mN/m [63]. Moreover, Jiang et al. found negatively charged MWCNTs to efficiently disrupt vesicles formed by positively charged lipids, involving both CNT binding and associated lipid extraction [64]. Similarly, Yi et al. reported that binding of carboxylated MWCNT to DOPC bilayers increased with increasing NaCl concentration, also to weakly negatively charged DOPC bilayers. In the presence of CaCl₂ binding was further promoted due to Ca²⁺ binding to the phosphate headgroups and resulting charge reversal. Despite this, liposome leakage results showed the MWNTs not to disrupt the DOPC bilayers on binding [65].

In one of very few studies of the effects of CNTs on lipopolysaccharides, Engel et al. investigated heterostructures of SWCNTs and iron oxide for bacterial inactivation, and found these to display high antimicrobial activity against E. coli. In order to investigate the mechanisms underlying this, E. coli mutant strains were compared to unmodified controls. Mutants with shorter LPS chains were found to be more sensitive to photocatalytic CNTs, indicating that LPS is less susceptible to oxidative degradation than phospholipids, acting as a protective shield against the photocatalytic nanomaterial and its oxidation products. This was further substantiated by results showing that inactivation of mutants with suppressed oxidative stress defense depended on oxidative stress [66].

3.4.3. Graphene

As nicely reviewed by Zhou et al., graphene variants are promising antimicrobial materials [67]. Addressing membrane interactions contributing to this, Liu et al. investigated the capacity of graphene oxide (GO) to attach to and disrupt DOPC bilayers. The kinetics of GO binding to DOPC bilayers was found to increase with increasing ionic strength, reflecting the importance of electrostatics for bilayer binding [68]. Investigating effects of lipid bilayer composition, Huang et al. reported that while zwitterionic DOPC liposomes remained intact after GO binding, cationic DOTAP-containing liposomes ruptured on GO binding [69]. Addressing the mechanisms underlying such graphene-induced membrane destabilization, Li et al. investigated the interaction of graphene with model lipid bilayers, as well as a different cell types [70]. Confocal and electron microscopy indicated edge-first uptake, as supported also by MD simulations. Related to this, Lu et al. investigated membrane interaction GO nanosheets as a function of the orientation the latter. In doing so, GO nanosheets were magnetically aligned and subsequently fixated by cross-linking of a surrounding polymer matrix. Exposing E. coli bacteria to such surfaces, GO nanosheets in vertical orientation were found to display higher antibacterial activity than GO in random and horizontal orientations. Mirroring this, GO nanosheets were found to disrupt DOPC vesicles. In addition to these direct particle effects, GO-induced oxidation of glutathione was observed, as was ROS generation. Both these mechanisms depend on membrane insertion, suggesting that the enhanced antibacterial activity of vertically aligned GO originates from an increased density of edges with an optimal orientation for membrane insertion and destabilization [71] (Fig. 6).

In line with this, Lv et al. applied particle dynamics simulations to analyze the energy involved in the indentation of graphene-covered atomic force microscopy (AFM) probes into DPPC bilayers. Again, vertically oriented graphene nanosheets were found to promote membrane disruption [72]. Similarly, Santiago et al. employed MD simulation to model the interaction modes of graphene with POPC/cholesterol liposomes and found a preference of membrane-inserted nanosheets for an orientation perpendicular to the bilayer surface [73]. In addition to such orientation effects, CNTs may influence the phase behavior of membranes. Exemplifying this, Mandal et al. reported on domain formation of lipid membranes in the presence of GO. In doing so, bilayer stacks of DPPC were formed and their structures investigated by X-ray reflectivity and diffraction. GO-loaded systems were found to display two sets of lamellar diffraction peaks, corresponding to GO-rich microdomains dispersed in a matrix of GO-poor phospholipids. In the GO-rich domains, GO flakes were found to penetrate into the hydrophobic bilayer core, while the GO-poor bilayers formed a structure closely related to the phase formed by lipid molecules alone [74].

3.4.4. g-C₃N₄

During the last few years, graphitic carbon nitride nanosheets (g-C₃N₄) have attracted considerable interest as photocatalyst due to these displaying potent oxidative degradation of a wide range of organics, as well as of bacteria and viruses. So far, however, very few studies have addressed the mechanisms underlying such effects. In one of only few studies addressing such mechanisms, Cui et al. investigated the effect of nitrogen-plasma-treatment of g-C₃N₄ (N-g-C₃N₄) on their antimicrobial effects against foodborne bacteria, and found bactericidal activities of >99% on N-g-C₃N₄ incubation in the dark, more than ten times higher than activities observed for g-C₃N₄. Furthermore, bacterial rupture was triggered by contact between g-C₃N₄ nanosheets and bacteria membranes, the latter also correlated to the abundance of surface defects and nitrogen vacancies in N-g-C₃N₄ [75].

4. Antimicrobial effects of photocatalytic nanomaterials

4.1. TiO₂-based nanoparticles

Among metal oxides, TiO₂ has been particularly extensively investigated as a photocatalytic material. For example, Molina-Reyes et al. compared *OH generation and antimicrobial effects of immobilized TiO₂ nanoparticles and nanotubes against E. coli. While similar *OH generation was observed for nanoparticles and nanotubes, the antibacterial activity was higher for TiO₂ nanotubes, inferred to be due to the relatively smooth surface of TiO₂ nanoparticles allowing closer surface contact compared to nanoparticle-based films [76]. Demonstrating the potent antimicrobial effects of TiO₂ under UV illumination, Arora et al. found potent antimicrobial effects of TiO₂ nanoparticles in mixed rutile/anatase against multi-drug resistant (MDR) P. aeruginosa, but only under UV illumination [30]. Analogously, Nica et al. found TiO₂ nanoparticles co-doped with iron and nitrogen to display potent antimicrobial and anti-biofilm activity, yet having low toxicity against lung and dermal cells [77]. In addition to such photocatalytic antimicrobial effects of TiO₂ alone, TiO₂ may be combined with antibiotics for potent antimicrobial effect against challenging pathogens. For example, Ahmed et al. investigated antimicrobial effects of TiO₂ nanoparticles on MDR P. aeruginosa. In doing so, twenty-five P. aeruginosa isolates were selected, which were completely resistant to cefepime (100%), or highly resistant to ceftriaxone (96%), amikacin (80%), or ciprofloxacin (76%). For all isolates, antibacterial activity of the TiO₂ nanoparticles was observed. Furthermore, TiO₂ nanoparticles combined with cefepime,
ceftriaxone, amikacin, and ciprofloxacin showed synergistic activity against all isolates [78].

In order to boost photocatalytic effects of TiO$_2$, several approaches have been developed, including: (i) doping (e.g., Co, Mn, Fe, I, S, N), (ii) deposition of silver halides (Cl, Br, I), (iii) coupling to noble metals (Pt, Pd, Ag) or semiconductors (e.g., ZnO, SnO$_2$, or CuO), and (iv) preparation of TiO$_2$ composites with polymers, organic photosensitizers, and other compounds. Through decreasing the band-gap and suppressing electron-hole pair recombination, such approaches result in enhanced ROS generation under visible light, in turn causing enhanced antibacterial effects [11]. Illustrating TiO$_2$ boosting by photosensitizers, Sulek et al. investigated porphyrins doped on TiO$_2$ nanoparticles with regards to their antimicrobial properties against _S. aureus_ and _E. coli_. The porphyrin-loaded TiO$_2$ nanoparticles were found to result in a 7-log reduction of _S. aureus_ under visible light illumination. The activity against Gram-negative _E. coli_ was significantly lower, but simultaneous addition of KI resulted in efficient inactivation also of the latter. The higher photocatalytic efficiency observed for Gram-positive _S. aureus_ was speculated to be due to differences in bacterial wall structure. In particular, the presence of LPS in Gram-negative _E. coli_ may decrease membrane permeability for lipophilic compounds and impede ROS penetration to reach targets within the bacterial cell [79].

Investigating the effect of metal ion doping in TiO$_2$ nanoparticles, Yadav et al. reported on enhanced photocatalytic activities of copper-, nickel- and iron-ion-doped TiO$_2$ nanoparticles under visible light against _E. coli_ and _S. aureus_ bacteria [9]. Furthermore, Sayilkan et al. reported on enhanced antibacterial activity of Sn$^{4+}$-doped TiO$_2$ against _E. coli_ and _S. aureus_ under illumination of weak UV light, compared to those displayed by undoped TiO$_2$ [80]. Analogously, Caballero et al. investigated Pt-doped TiO$_2$ against _E. coli_ under fluorescent light illumination, and found this to display excellent antimicrobial activity due improved charge separation in the doped nanomaterial [81].

Boosting the activity of TiO$_2$ through formation of heterostructures with noble metal nanoparticles, Xu et al. investigated TiO$_2$ nanotubes as scaffolds for layer-by-layer deposition of AuNPs. Due to surface plasmon resonance of AuNPs, the coated TiO$_2$ nanotubes were found to display improved photocatalytic activity under visible light, as well as high antibacterial activity against _Porphyromonas gingivalis_ and _Fusobacterium nucleatum_ and high anti-inflammatory efficiency. In contrast, non-decorated TiO$_2$ nanotubes did not display any substantial such effects (Fig. 7) [82]. Similarly, Viet et al. found AgNPs to improve photocatalytic and antibacterial activities of TiO$_2$ nanotubes. Quantitatively, AgNP-coated TiO$_2$ nanotubes at 20 ppm were found to eliminate 99.99% of _S. aureus_ after 60 min under sunlight illumination [83]. Similarly, Zheng et al. investigated AuNP-coated TiO$_2$ nanotubes. The latter displayed pronounced photocatalytic activity under visible light.
irradiation, but also high ROS yield due to suppression of e\textsuperscript{-}·h\textsuperscript{+} recombination, as well as surface plasmon resonance of the AuNPs. As a result of this, the antibacterial efficacy against a multi-species biofilms depended on the amount of AuNPs decorating the TiO\textsubscript{2} nanotubes. Furthermore, experiments in New Zealand white rabbits indicated promoted soft tissue healing for the AuNP-decorated TiO\textsubscript{2} nanotubes [84]. Also related, Liu et al. reported on composite nanoparticles, prepared by self-assembly of TiO\textsubscript{2} and V\textsubscript{2}O\textsubscript{5}, and found these to display excellent antibacterial properties against \textit{E. coli} under light irradiation due to generation of hydroxyl radicals (\textsuperscript{•}OH) [85].

4.2. Metal nanoparticles

Among metal nanoparticles, AgNPs generally display the highest antimicrobial activity, increasing with decreasing particle size [86–88]. In addition to particle-mediated membrane destabilization, AgNPs reach antimicrobial effects through release of Ag\textsuperscript{+}, which have an affinity to sulphydryl groups located in bacterial cell walls, interfering with the electron transport chain through the cell wall porins. In addition, Ag\textsuperscript{+} may complex with various cytoplasm components, as well as with DNA and RNA [89]. Demonstrating the importance of released Ag\textsuperscript{+}, Wirth et al. investigated colonization by \textit{Pseudomonas fluorescens} on AgNP-covered surfaces, and found that the ability of surfaces coated with such nanoparticles to inhibit bacterial colonization was controlled by dissolved Ag\textsuperscript{+} in the system [90]. On the other hand, illustrating the importance of direct nanoparticle destabilization of bacterial membranes for antimicrobial effects, Feng et al. investigated AuNP binding to, and resulting lysis of, \textit{Shewanella oneidensis} and \textit{Bacillus subtilis} (\textit{B. subtilis}), using either anionic (mercaptopropionic acid), cationic (mercaptopropylamine), or cationic/polyelectrolyte (poly(allylamine hydrochloride)) surface modifications. It was found that both cationic AuNPs, but particularly so the cationic polyelectrolyte-coated ones, were potently antimicrobial to both bacteria, which correlated to nanoparticle binding [91].

In one of relatively few studies of photocatalytic antimicrobial activities of metal nanoparticles alone (as opposed to such materials employed in combination with other photocatalytic nanomaterials), An et al. reported that potent antibacterial effects can be obtained by combining the photosensitizer [Ru(bpy)\textsubscript{3}\textsuperscript{2+}] with AgNPs. Formation of the hybrid nanoparticles was achieved through employing self-assembled DOPS/DPPC/cholesterol membrane, which coated the AgNPs and bound the photosensitizer. Upon illumination, plasmon-enhanced photoexcitation triggered lipid oxidation and membrane destabilization. Photooxidation was found to induce the release of oxidized lipids, [Ru(bpy)\textsubscript{3}]\textsuperscript{2+}, and Ag\textsuperscript{+}, which interacted synergistically for antimicrobial effects. Employing this approach, a 7-log decrease was observed for Gram-positive \textit{Arthrobacter sp.} and a 4-log decrease for Gram-negative \textit{E. coli} on visible light illumination for 1 h [92].

4.3. Quantum dots

As with other types of nanoparticles, QDs may reach antimicrobial effects through direct membrane destabilization. Exemplifying this, Lai et al. investigated antimicrobial effects of CdTe/ZnS QDs modified with either anionic mercaptpropionic acid or cationic cysteamine against \textit{E. coli}. While microcalorimetry showed the positively charged particles to be more potently antimicrobial than the negatively charged ones, fluorescence anisotropy showed the positively charged particles to be more potently antimicrobial than the negatively charged ones, fluorescence anisotropy showed the former to result in increased fluidity and permeability of \textit{E. coli} and DPPC membranes [93]. Furthermore, Chen et al. studied antimicrobial properties of ZnO
nanoparticles coated by the cationic antimicrobial peptide UBI29-41, the latter facilitating membrane binding and destabilization, and found these to enhance the antimicrobial effect of vancomycin against *B. subtilis* and *S. aureus* [94].

Owusu et al. reported on the antimicrobial activity of indium-based QDs against methicillin-resistant *S. aureus* (MRSA) and a carbapenemase-producing strain of *E. coli*. In order to promote generation of oxygen and superoxide radicals (*O$_3^-$*), QDs were incorporated into a polyurethane matrix with the photosensitizer crystal violet for close proximity between the QD and the dye. Through this, potent antibacterial activity was observed under visible light against both antibiotics-resistant strains. In addition, strong inhibition of bacterial killing was observed on addition of the superoxide scavenger superoxide dismutase [95]. Analogously, Liu et al. reported that Co-doped ZnO QDs allowed efficient separation of photogenerated electrons and holes, resulting in high ROS generation, notably hydroxyl radicals (·OH) and superoxide anions (·O$_2^-$). This caused the antibacterial activity of Co-doped ZnO QDs to be drastically improved compared to pristine ZnO QDs. Furthermore, smaller-sized Co-doped ZnO QDs were inferred to favor bacteria contact, thereby further boosting antibacterial activities [96].

Similarly, Karami et al. investigated the antimicrobial activity of ZnO nanoparticles doped with I and Ag against *S. aureus* and *E. coli*. The double-doped nanoparticles displayed potent efficacy against both types of bacteria, depending on both ROS generation and Ag$^{+}$ release [97].

Liu et al. investigated TiO$_2$/AgVO$_3$ QD composites, where AgVO$_3$ were bound on the surface of TiO$_2$ nanoparticles. The TiO$_2$/AgVO$_3$ composite was found to display high and stable visible light antimicrobial activity against *E. coli*, which relied on (i) efficient distribution of AgVO$_3$ QDs, (ii) enhanced light harvesting, and (iii) improved separation efficiency of electron-hole pairs [98]. Similarly, Ma et al. investigated CdSe QDs/graphene/TiO$_2$ composites. Through graphene, these displayed extended visible-light absorption, while TiO$_2$ promoted efficient charge separation. As a result, CdSe QDs/graphene/TiO$_2$ composites efficiently generated photocatalytic species, such as $h^+$, ·OH, and ·O$_2^-$, as confirmed by transient photocurrent responses, scavenger addition, and detection of ·OH radicals [99].

Illustrating the theranostic potential of QDs, Kuo et al. investigated graphene QDs as photosensitizers and bioimaging agents. The QDs were found to efficiently generate ROS through two-photon excitation, resulting in potent antimicrobial effects against both Gram-negative and Gram-positive bacteria. In addition, the QDs can act as contrast agent for imaging bacteria or other infection biomarkers during or after phototoxic therapy. Particularly for MDR bacterial strains, such theranostic opportunities are highly interesting, as they reduce the risk of prematurely stopping treatment [100].

### 4.4. Carbon-based nanomaterials

#### 4.4.1. Fullerences

Investigating the role of particle-mediated membrane destabilization, Huang et al. compared the partitioning of fullerences into bacterial membranes with their antimicrobial effects [101], and found a good correlation for Gram-negative *P. aeruginosa* and *E. coli*, as well as Gram-positive *S. aureus*. In addition to the importance of direct particle-mediated membrane destabilization thus demonstrated, however, antimicrobial effects were substantially enhanced by photocatalytic effects. However, it is interesting to note that Lyon et al. reported nC60 to cause oxidative inactivation to bacteria also in the absence of ROS, as inferred from results on membrane potential, protein oxidation, and suppression of respiration [102]. Demonstrating the translation to the situation in vivo, Lu et al. found that dimethylpyrroolidinum-functionalized C$_60$ to induce broad-spectrum antimicrobial activity on illumination [103]. For excisional wounds in mice, infected with either *P. aeruginosa* or *Proteus mirabilis* (*P. mirabilis*), illumination in the presence of C$_60$ resulted in bacteria suppression, an effect not found in the control groups. Moreover, an 82% survival compared was observed, compared to 8% survival with no treatment.

Zhang et al. employed peptide-modulated self-assembly of fullerences in order to boost their photocatalytic efficiency. Results obtained both in vitro and in vivo showed the fullerene-based hydrogels to potently suppress MDR *S. aureus* and accelerate wound healing [104]. Analogously, Zhang et al. investigated amine modified fullerene (C$_{60}$-(ethylenedi-imine)$_n$-C$_{70}$-EDA)$_n$, and found this to display potent antimicrobial effects against MDR *E. coli*, while simultaneously displaying low toxicity against human epidermal keratinocyte-adult (HEK-a) cells. In addition, C$_{70}$-EDA$_n$ was found to efficiently suppress bacterial infection in vivo, and to accelerate wound healing through immune modulation and growth factor triggering [105].

#### 4.4.2. Carbon nanotubes

In part due to direct “particle”–based membrane destabilization, CNTs may be potentially antimicrobial. Illustrating this, Zardini et al. studied antimicrobial effects of MWCNTs modified with cationic lysine or arginine, and found the antimicrobial potency to be significantly higher for the cationically modified MWCNTs, and particularly so for arginine, demonstrating the importance of electrostatics for bacterial membrane destabilization by MWCNTs [106]. Analogously, Aslan et al. modified SWNTs through layer-by-layer deposition, terminated with a cationic poly(L-lysine) layer, and found more potent antimicrobial effects against *E. coli* and Staphylococcus epidermidis (*S. epidermidis*) for cationic than for pristine SWNTs [107]. In addition to such direct membrane destabilization, however, CNT-mediated oxidation plays a key role as well. Thus, employing similar-dimension SWCNTs with different levels of defects, Vecitis et al. found antimicrobial effects against *E. coli* to be accentuated with an increasing fraction of metallic SWCNTs. In parallel, glutathione oxidation and morphological changes both depended on the electronic structure of the SWCNTs. From this, bacterial inactivation was proposed to occur through SWNT/bacteria binding, followed by bacteria membrane destabilization and oxidative degradation (the latter depending on SWCNT electronic structure) [108].

CNTs have also been combined with photosensitizers, as well as with other types of antimicrobial nanoparticles for boosted antimicrobial performance. For example, Parasuraman et al. investigated antibacterial and antibiofilm activities of CNTs coated with the organic photosensitizer Methylene Blue, and found 4.86-log and 5.55-log reductions for *E. coli* and *S. aureus*, respectively. Mirroring this, biofilm assays showed higher inhibition for *S. aureus* than for *E. coli*. Based on results from cytoplasmic protein leakage and confocal microscopy, the bacterial membrane was inferred to be the primary target for photocatalytic degradation, while the higher inactivation observed for *S. aureus* was suggested to be due to higher ROS levels inside these bacteria due to barrier properties provided by LPS in Gram-negative bacteria [109].

In another approach, Huang et al. studied antibacterial activities of CuNP-loaded MWCNTs in the presence H$_2$O$_2$ and found potent suppression of *E. coli*, caused by a combination 0·OH formation and Cu$^{2+}$ release mediated by MWCNT-promoted electron transfer [110]. Similarly, Yallappa et al. found that MWCNTs functionalized by CuNPs and AgNPs efficiently suppressed *S. aureus*, Salmonella typhi, *E. coli*, and *P. aeruginosa*, while showing low toxicity against epithelial cells [111]. Improving the performance of such materials further, Chaudhari et al. conjugated silver-coated SWCNTs with the antimicrobial peptide TF359, and found peptide conjugation to increase membrane binding and antimicrobial effects, yet rendering low toxicity towards Hep2 cells and murine macrophages [112]. Furthermore, Abbas et al. reported on antimicrobial effects against *E. coli* and toxicity against HaCaT cells for TiO$_2$-loaded CNTs. In the absence of light, the antimicrobial activity of TiO$_2$ increased after CNT binding, likely due to antimicrobial effects of the CNTs themselves, and to reduced TiO$_2$ aggregation after CNT immobilization [113]. In line with the latter observation, Mohammad et al. investigated SWCNTs and acid-treated MWCNTs, coated with Ag-doped TiO$_2$ nanoparticles. MWCNTs-Ag/TiO$_2$ and SWCNTs-Ag/TiO$_2$
both have lower band gap than pristine TiO₂, promoting ROS generation on illumination. The materials were found to be strongly antibacterial against both Gram-positive *S. aureus* and Gram-negative *E. coli*, although quantitatively, Gram-negative *E. coli* bacteria were again found to be more resistant to UV illumination than Gram-positive *S. aureus* [114].

### 4.4.3. Graphene

The antimicrobial activity of layered carbon-based nanomaterials, Liu et al. found the antibacterial activity of such materials against *E. coli* to decrease in the order graphene oxide (GO) > reduced graphene oxide (rGO) > graphite (Gr) and graphite oxide (GO), and that this was mirrored in bacterial cell lysis [115]. On a related theme, Liu et al. found larger GO sheets to more efficiently suppress *E. coli* than smaller ones [116]. This was found not to be due to differences in aggregation or oxidation capacity. Instead, AFM results showed large GO sheets to cover bacteria more efficiently, while small GO sheets are less efficient in covering and isolating bacteria cells from their surrounding.

Illustrating photocatalytic effects of such materials, Zhang et al. investigated antimicrobial effects of graphene (GDY) and graphdiyne oxide (GDYO). In particular, surface oxidation was found to provide inert GDY with potent antibacterial activities against *S. aureus* and *E. coli*, an effect related to increased ROS levels under visible light irradiation [117]. Furthermore, Xia et al. investigated bacterial inactivation by graphene coated by plasmonic Ag/AgX under visible light illumination. The improved antibacterial effects observed for such heterostructures were attributed to suppressed charge recombination, as well as boosted ROS formation and Ag⁺ release [118]. Along the same line, Wang et al. reported on mesoporous silica nanoparticles loaded with graphene quantum dots (GQDs) and ethylenimine (EM). Bacterial density experiments confirmed that such modified GQDs displayed good antimicrobial effects against *S. aureus* and *E. coli* under irradiation caused by photocatalytic oxidation. Furthermore, healing of bacteria-infected wounds in mice confirmed these particles to display good therapeutic effect, characterized also by significantly reduced inflammatory factors in blood [119]. Analogously, Mei et al. investigated oligomeric chitosan-functionalized graphene dots and their use under for promoting rapid healing of bacteria-infected wounds under visible light illumination. The graphene dots generate ROS and heat under illumination, while the positively charged chitosan promotes bacteria binding and membrane lysis. In contrast to the pronounced antimicrobial effects, the hybrid nanoparticles were found to display low toxicity against erythrocytes and RAW264.7 cells, and to improve the healing of *S. aureus*-infected wounds [120]. Similarly, Prema et al. found ZnO-decorated GO to potently suppress *E. coli*, *Salmonella typhi*, *P. aeruginosa* and *Shigella flexneri* under visible light illumination. ROS, lactate dehydrogenase leakage, and DNA fragmentation quantification showed bacterial inhibition to be caused by ROS-mediated membrane oxidation and integrity loss, not by DNA fragmentation [121].

### 4.4.4. Carbon nanodots

Carbon dots (CQDs) have recently attracted considerable interest due to their effective visible light-activated antimicrobial activities toward bacteria. For example, Li et al. found CQDs from vitamin C to display broad-spectrum antimicrobial activity. The origin of these effects were found to be due to several mechanisms operating in concert, including lysis of bacterial walls, perturbation of DNA/RNA, and inhibition of gene expression [122]. Similarly, Nie et al. reported 99.9999% suppression of both *E. coli* and *S. aureus* by CQDs under visible light illumination. After illumination, fragmented cell membranes were observed for bacteria incubated with CQDs, while ROS quantification indicated that bacteria were inactivated by singlet oxygen, and not by superoxide or hydroxyl radicals [123].

CQDs are strongly absorbing in the visible region, triggering rich excited state processes. Investigating the consequences of these, Dong et al. coupled spectroscopic probing of these processes to evaluation of photoinduced antibacterial effects of CQDs. The potent efficient antibacterial activities of the CQDs observed against *Enterococcus* spp. and *Enterococcus faecium* on illumination were attributed to photo-generated e⁻ and h⁺ trapped at surface defect sites, and subsequent ROS formation. Interestingly, the separated redox species in CQDs could not be quenched by ROS scavengers, thus rendering light-activated CQDs potent antimicrobial agents [124].

As for other photocatalytic nanomaterials, the light-induced oxidative capacity of CQDs is influenced by surface coating. Illustrating this, Mandal et al. reported on amplification of visible light-induced ROS generation in CQDs conjugated with bovine serum albumin (BSA). BSA modification was found to result in shifted excitation maxima and increased ROS generation. In addition, conformational changes of BSA upon CQD conjugation decreased its susceptibility to (bacteria-associated) pepsin degradation. As a result of these effects, BSA-conjugated carbon dots displayed potent suppression of both *E. coli* and *S. aureus* [125]. Somewhat related, Kovacova et al. investigated CQD/polyurethane composites, and found these to be effective in generating singlet oxygen upon blue light illumination. This caused a 5-log suppression of both *S. aureus* and *E. coli* within 60 min of irradiation.

Despite this, the composite nanoparticles displayed low toxicity against mouse embryonic fibroblasts, moderate toxicity against adenocarcinomic human epithelial cells, and minor hemolysis [126]. Analogously, Marcovic et al. investigated CQD/polydimethylsiloxane surfaces, employing luminescence and electron paramagnetic resonance spectroscopy to demonstrate that blue light irradiation generated singlet oxygen. As a result of this, the nanocomposite efficiently killed *S. aureus*, *E. coli* and *Klebsiella pneumoniae* only a 15 min of irradiation. In contrast, the composite surface was not toxic towards murine NIH/3 T3 cells [127].

### 4.4.5. g-C₃N₄

Due to its wide absorption spectrum, g-C₃N₄ generates hydroxyl and superoxide radicals under visible light, causing potent antimicrobial effects. Investigating this, Yadav et al. reported bacterial inhibition levels of ~90% for *S. aureus* and ~99% for *E. coli* by g-C₃N₄ at a 0.1 mg/mL. This light-induced bacteria toxicity was inferred to be caused by a combination of direct nanoparticle-induced membrane destabilization and ROS generation under light illumination. Contrasting the antimicrobial effects, g-C₃N₄ nanoparticles were low-toxic to fibroblasts up to 3.2 mg/mL. Despite such promising results, however, g-C₃N₄ suffers from several shortcomings, which prevent it from reaching its full potential as in antimicrobial photocatalysis. These include, e.g., high recombination rate and poor light absorption above 460 nm. Considering this, as well as aspects related to membrane interactions, modification of g-C₃N₄ for optimized photocactivity has recently attracted considerable interest. Exemplifying this, Cui et al. investigated nitrogen-plasma-treated g-C₃N₄ nanosheets (N-g-C₃N₄) and found these to display bacterialicidal rates of >99% for a range of foodborne bacteria after 8 h of incubation in the dark, rates 10 times higher than those for pristine g-C₃N₄. The antimicrobial effects were found to be caused primarily by bacterial membrane lysis on direct contact with g-C₃N₄ nanosheets [129]. In addition, such surface modifications frequently influence also photocatalytic effects. For example, Zeng et al. studied C₃N₄-coated with cationic polyethyleneimine (PEI), and found the surface modification to significantly improve light-induced generation of long-lived ROS (O₂ - and H₂O₂) compared to C₃N₄. Additionally, scanning electron microscopy (SEM) and AFM showed an increase of close bacteria-photocatalyst contact by electrostatic adhesion. As a result, the inactivation activity C₃N₄ under illumination was boosted after PEI modification.

In another approach for boosting the photocatalytic performance, Yu et al. investigated antimicrobial effects of AgBr-modified g-C₃N₄ photocatalysts against *E. coli*, and found these to display potent bacteria...
inactivation. Quantitatively, a 4.80-log suppression was observed after 150 min of visible light irradiation in the presence of AgBr-modified g-C₃N₄. This was a 4.2-log improvement compared to pristine g-C₃N₄. The boosted photocatalytic activity was found to be due to a reduced band gap, resulting in effective production and transfer of photo-induced electrons under visible light irradiation. Furthermore, photogenerated h⁺ and ROS were shown to damage bacterial membranes and to destroy metabolic processes, resulting in leakage of K⁺ and proteins, lipid oxidation, degradation of intracellular protein, and a reduction of the ATP levels (Fig. 8) [131]. Similarly, Ai et al. investigated γ-Fe₂O₃/Ag/AgCl/g-C₃N₄ heterostructure nanoparticles and found these to display efficient E. coli suppression under visible-light illumination. Scavenging experiments revealed *OH and H₂O₂ to be most important for photocatalytic bacterial inactivation. In this heterostructure, the conduction and valence band potentials of γ-Fe₂O₃, Ag, AgCl, and g-C₃N₄ allowed continuous transfer of photogenerated electrons, thereby suppressing recombination of electron-hole pairs and resulting in enhanced antimicrobial activity under illumination [132]. Somewhat related, Gao et al. investigated Fe⁺⁺-doped C₃N₄ and found potent suppression of P. aeruginosa (99.9986%), E. coli (99.9974%), and S. aureus (99.9876%) after 300 min under visible-light irradiation. Radical trapping experiments showed the superoxide radical (O₂⁻⁻) to be the ROS of primary importance. In addition, through Fe³⁺ doping and the Fenton reaction, hydroxyl radicals (*OH) were generated to further enhance the photocatalytic performance of the heterostructure [133].

Related to nanoparticle heterostructure formation by g-C₃N₄, Wu et al. investigated AgNP/polydopamine (PDA)/g-C₃N₄ nanoparticles and found these to potently suppress E. coli, with a minimum inhibition concentration (MIC) of 9.5 ppm and a minimum bactericidal concentration (MBC) of 6.3 ppm. Despite this, toxicity against human umbilical vein endothelial cells (HUVECs) was low. In addition, PDA resulted in good nanoparticle stability, with only 0.18% of Ag⁺⁺ detected after 30 days. The antibacterial performance was inferred to be due to a combined effect of photocatalytic PDA-modified g-C₃N₄ and direct action of AgNPs. Under illumination, the surface plasmonic effect of AgNPs and the incorporation of PDA were found to enhance the photocatalytic activity of g-C₃N₄, resulting in formation of *OH and *O₂⁻⁻. Of these, particularly *OH together with h⁺, can boost antibacterial effects through oxidative degradation of lipid membranes and other bacterial components [134]. Somewhat related, Ding et al. investigated CuS/g-C₃N₄ heterostructure nanoparticles. These were found to display enhanced photocatalysis under visible light compared to CuS or g-C₃N₄ separately due to efficient charge separation. In addition, the hybrid nanoparticles displayed photothermal effects, increasing with their CuS content. At high CuS content, however, the photocatalytic effects were found to decrease again as a result of increased charge recombination. At a CuS content of 20%, maximal combined effects of photothermal action and photocatalysis under light irradiation was observed, resulting in ~99% inactivation of both S. aureus and E. coli after 1 h of illumination [135].

Focusing on heterostructures between g-C₃N₄ and metal oxide nanoparticles and QDs, Wang et al. investigated AgVO₃ QDs interdispersed g-C₃N₄, and found the hybrid material to be able to efficiently inactivate Salmonella H9812. Due to efficient ROS production, the bactericidal suppression by AgVO₃ QDs/g-C₃N₄ was >96% at 0.75 mg/mL after 10 min of visible-light illumination. Live/Dead assay and membrane potential results on membrane integrity, as well as SEM, PCR and protein content assays to verify leakage of bacterial cell content, all indicated that ROS generation resulted in destabilization of bacterial cell membranes [136]. Analogously, Wu et al. investigated heterostructures formed by MnO₂ and g-C₃N₄ at metallic Ti implants. In this structure, MnO₂ favors transfer and separation of charges, resulting in enhanced photoconversion. As a result, ROS generation was increased, allowing oxidative degradation of bacterial lipids, protein and DNA. In addition, glutathione, which counteracts oxidative stress in bacteria, was oxidized by MnO₂ after ROS-mediated membrane destabilization. As a result of this, MnO₂/g-C₃N₄ displayed >99% suppression of both S. aureus and E. coli after 20 min of illumination [137]. Analogously, Xiang et al. investigated a ZnO/QDs/g-C₃N₄ Z-scheme heterojunction, aimed at killing bacteria under visible light irradiation due to combined photothermal and photothermal effects. In this system, the QDs bridged g-C₃N₄ and ZnO, effectively suppressing charge recombination, and enhancing the photocatalytic effects. In addition, QDs provided excellent photothermal properties to the hybrid material. As a result of

---

Fig. 8. AgBr-modified g-C₃N₄ photocatalysts display potent antimicrobial effects against E. coli, reaching up to 4.80-log under 150 min of visible light irradiation, a 4.2-log improvement compared to that of pristine g-C₃N₄ under the same experimental conditions. The boosted photocatalytic activity of AgBr/g-C₃N₄ is due to effective production and transfer of photo-induced electrons under visible light irradiation. Photogenerated h⁺ and ROS damage bacterial membranes and destroy metabolic processes, resulting in leakage of potassium ions and proteins (A), lipid peroxidation (B), degradation of extracellular protein (B), and a reduction of the ATP levels (C), which finally lead to bacterial death. (Redrawn from [131].)
these combined effects, ZnO/CDs/g-C₃N₄ suppressed S. aureus and E. coli by 99.97% and 99.99%, respectively, after 15 min of visible light illumination. In addition, released Zn²⁺ ions promoted fibroblast growth, thereby accelerating the wound healing process [138]. Similarly, Gao et al. investigated heterostructures composed of sulfonated GO (SGO), ZnO, and AgNPs (SGO–ZnO–AgNP). Under visible illumination, the photocatalytic antimicrobial effect of SGO–ZnO–AgNP against E. coli was found to be superior to those of ZnO, SGO–ZnO, and ZnO–AgNP, which was attributed to: (i) beneficial effects on charge separation by SGO, (ii) enhanced light absorption due to surface plasmon resonance of AgNP, and (iii) pronounced light scattering due the hierarchical structure of the composite [139].

5. Antiviral effects of photocatalytic nanomaterials

Compared to bacteria, viruses have a lower infectious onset of <10–10⁵ particles and a higher illness risk of 10–10,000 times under similar exposure [140]. For example, exposure to a single rotavirus particle has been found to translate to a 31% chance of infection [141]. Considering this, it is of vital importance to identify approaches for reaching the low viral infection thresholds. Of relevance to the present overview, several organic photosensitizers have demonstrated good potential for viral inactivation, including phthalocyanines, merocyanines, porphyrin derivatives, and Methylene Blue. However, such photosensitizers also suffer from several shortcomings, such as poor solubility and related problems with stability and delivery to viral infections sites [13]. Considering this, photocatalytic nanoparticles are attracting increasing interest for oxidative antiviral effects.

5.1. TiO₂-based nanoparticles

As with bacteria, TiO₂-based nanomaterials are among the most widely investigated for antiviral effects. For example, Sato et al. reported the effect of crystalline structure on the antiviral activity of TiO₂ particles [142]. In particular, a 70/30 anatase/rutile mixture was found to display maximum viral inactivation, which was 2 and 11 times higher than that of pristine anatase and rutile, respectively. The boosted antiviral effect was attributed to contact between two types of TiO₂ particles, promoting ROS generation. Furthermore, Daikoku et al. investigated ceramic substrates coated with TiO₂ nanoparticles and found these to be efficient in inactivating airborne influenza virus [143]. Investigating the effect of light intensity for nanoparticle-induced photocatalysis, Ishiguro et al. applied low-intensity UVA illumination for inactivation of Qβ bacteriophages by TiO₂ films. As expected, only a small fraction of phages was inactivated at a very low light intensity (0.001 mW/cm²). With increasing light intensity, phage inactivation increased significantly, although increased charge recombination eventually resulted in photoenergy dissipation [144].

Comparing photocatalytic inactivation between bacteria and viruses, Cho et al. and found bacteriophage MS2 to be more resistant than E. coli bacteria to oxidative degradation by TiO₂ under illumination. This was speculated to be due to the rigidity of the viral protein shell, necessitating intensive oxidation for destabilization [145]. Furthermore, Misteer et al. reported bacteriophage resistance to photocatalytic inactivation by TiO₂ to decrease in the order of PK772 > 4X174 > MS2 [146]. In contrast Mac Mahon et al. observed resistance as 4X174 > PK772 > MS2 for Ru(bpy)₃Cl₂ under otherwise identical conditions, suggesting that viral susceptibility to photocatalytic degradation varies with the type photocatalyst [147]. This illustrates the complexity of the issue of viral spectrum width, and emphasizes the need for further mechanistic studies.

The presence of ions may strongly affect the antiviral potency of TiO₂. For example, Fe³⁺ has been observed to increase inactivation of MS2 by TiO₂ under UV illumination due the Fenton reaction [148]. In contrast, Na⁺, Cl⁻, and Br⁻ have all been reported to have small effects, while K⁺, Ca²⁺, NO₃⁻, SO₄²⁻, and PO₄³⁻ have been found to suppress viral inactivation [149]. Furthermore, Zhang et al. found both Na⁺ and Ca²⁺ to promote viral inactivation of MS2 by g-C₃N₄ under visual light, an effect speculated to be due to these ions being able to coordinate with negatively charged nitrogen in g-C₃N₄ for improved charge separation [150].

As for antibacterial effects, photocatalytic inactivation of viruses can be boosted by formation of heterostructures between TiO₂ and noble metal clusters or nanoparticles. For example, Miyauchi et al. investigated copper oxide nanoclusters grafted TiO₂ (CuO/TiO₂). The CuO nanoclusters consist of both Cu(I) and Cu(II), where Cu(I) provides antiviral effects also under dark conditions. Moreover, Cu(II) acts as electron acceptor in interfacial charge transfer, resulting in the formation of antiviral Cu(I) species, as well as h+ with strong oxidation power under visible-light irradiation. Employing these systems, potent antiviral effects were observed against influenza virus A and bacteriophage Qβ [151]. Furthermore, Liga et al. investigated AgNP-doped TiO₂ and found their antiviral effect against MS2 to increase with increasing Ag content. Due to surface plasmon resonance generated by the AgNPs, visible light photocatalysis was promoted. As a result, AgNP/TiO₂ strongly enhanced photocatalytic disinfection by: (i) enhancing *OH generation, and (ii) promoting close contact between the virus and the photocatalyst [152].

Analogously, photocatalytic inactivation of viruses can be boosted by formation of heterostructures between TiO₂ and transition metals. For example, Kim et al. employed Pd-TiO₂ and vacuum UV to remove airborne MS2, reaching nearly 90% removal at an illumination time of only 0.009 s [153], whereas Choi et al. reported >99% inactivation of H1N1 influenza virus for TiO₂ doped with Mg, Fe, and Mn [154]. Related to this, Zheng et al. investigated photocatalytic Cu-TiO₂ nanofibers, and found these to provide a 5-log inactivation of phage f2 viruses under visible-light irradiation [155].

5.2. Metal nanoparticles

As with bacteria, studies of antiviral effects of metal nanoparticles have focused primarily on the combination of such nanomaterials with other photocatalysts (as discussed throughout this review), while studies on photocatalytic antiviral effects of metal nanoparticles alone are more scarce. Exemplifying the latter, however, Kim et al. investigated porous AuNP and their antiviral properties. The porous AuNPs were found to strongly suppress viral infectivity of MDCK cells to H1N1, H3N2, and H9N2 viruses. In addition, viability of infected cells increased to 96.8% after nanoparticle treatment, compared to 33.9% without treatment. Intracellular viral RNA quantification furthermore showed the nanoparticles to block the viral entry process through inducing conformational changes of hemagglutinin [156].

5.3. Carbon-based nanomaterials

5.3.1. Fullerenes

In an early investigation, Käsemann et al. investigated singlet-oxygen-generating C₆₀ and its antiviral effects against Semliki Forest Virus and Vesicular Stomatitis Virus. On visible light illumination for 5 h, infectivity was suppressed by more than 7-logs. This viral inactivation was found to depend on oxygen, but to be independent of presence of plasma proteins [157]. As with bacteria, surface modification of fullerenes can be employed to boost photocatalytic inactivation of viruses. Illustrating this, Cho et al. investigated C₆₀ with different functional groups (NH₂, COOH, OH), all showing higher photocactivity than fullerol under UV irradiation. Cationic amino-modified C₆₀ exhibited particularly efficient inactivation of MS2, likely through electrostatic attraction promoting close contact between positively charged C₆₀ and negatively charged MS2 viruses [158]. Interactions promoting photocatalytic virus inactivation seem to go beyond electrostatics, however, as Kraevaya et al. reported on C₆₀ fullerene derivatives decorated with phosphonic acid residues. Self-assembly of these compounds in aqueous
solution was found to result in the formation of nanostructures with potent and broad-spectrum antiviral activity [159]. Furthermore, entry of both Zika (ZIKV) and Dengue (DENV) viruses into host cells is mediated by receptors, including DC-SIGN. Considering this, blocking this receptor through multivalent glycoconjugates has been suggested as an approach for inhibiting the infection process. Addressing this, Ramos-Soriano et al. investigated tridecafullerenes conjugated with 1,2-mannobiosides. These systems were tested against ZIKV and DENV, and found to potent antiviral activity in the picomolar range [160].

5.3.2. Graphene

Donskyi et al. reported graphene derivatives with polyglycerol sulfate and fatty amine conjugations against simplex virus type 1 (HSV-1). Such derivatives were found to display particle-induced antiviral effects by binding to the virus surface through electrostatic and hydrophobic interactions. While C_{12}-functionalized graphene showed the highest antiviral activity, it also displayed cell toxicity against mammalian Vero cells. In contrast, the C_{6} and C_{9}-conjugates showed low toxicity against Vero cells but still potent suppression of HSV-1 [161]. Despite promising results of this type for graphene and GO, these are prone to aggregation, limiting their antiviral efficiency. In an effort to address this, Hu et al. immobilized aptamers to GO. Due to its improved colloidal stability, as well as its promoted virus binding, the resulting composite was found to more efficiently inactivate bacteriophage MS2 via oxidative damage of viral proteins and nucleic acids under visible-light illumination [162].

Another approach for boosting antiviral effects of graphene is through formation of heterostructures with metal and semiconductor nanoparticles. Exemplifying this, Akhavan et al. investigated graphene-WO_{3} films, and found these to dramatically suppress (log-5) MS2 after 3 h of visible-light irradiation. Furthermore, results from mechanistic studies showed that the protein capsid was degraded first, and RNA subsequently released [163]. For MS2 capsids, the coat and the maturation A proteins have been demonstrated to be detrimentally affected by GO-aptamer [162] and G-WO_{3} [162], while g-C_{3}N_{4} has been demonstrated to have analogous effects on the maturation A and the replicase proteins [164].

5.3.3. g-C_{3}N_{4}

Zhang et al. investigated the antimicrobial effects of g-C_{3}N_{4} for E. coli bacteria and human adenovirus in the presence of H_{2}O_{2} under visible light irradiation. While pristine g-C_{3}N_{4} caused a 5-log reduction of E. coli bacteria within 150 min, the activity against human viruses was minute (<0.5-log). In the presence of oxidants, however, a greatly enhanced antimicrobial activity was observed for both E. coli bacteria (5-log reduction within 120 min) and human adenovirus (2.6-log reduction within 150 min) (Fig. 9) [165]. Furthermore, Li et al. showed g-C_{3}N_{4} to display visible-light photocatalytic antiviral activity against MS2, significantly better than N-TiO_{2} and Bi_{2}WO_{6}, and comparable to Ag@AgCl. However, 300 min was needed for complete (8-log) eradication of bacteriophage MS2 [164]. To further boost the antiviral activity of g-C_{3}N_{4}, Zhang et al. investigated heterostructures formed by oxygen-doped graphitic carbon nitride (O-g-C_{3}N_{4}) coated with

![Fig. 9. Comparison of photocatalytic deactivation bacteria (E. coli) and viruses (human adenovirus) by g-C_{3}N_{4} in the presence of H_{2}O_{2} under visible light illumination. While pristine g-C_{3}N_{4} was found to achieve a 5-log reduction of E. coli bacteria within 150 min (A), it displayed negligible activity against human viruses and spores (<0.5-log) (B). By addition of oxidants, however, a greatly enhanced antimicrobial activity was observed for both E. coli bacteria (5-log reduction within 120 min) (C) and human adenovirus (2.6-log reduction within 150 min) (D). (Redrawn from [165]).]
hydrothermal carbonization carbon (HTCC). The heterostructures displayed strong visible light absorption, resulting in higher antiviral activity against human adenovirus type 2 (HAdV-2) under visible light illumination, compared to HTCC, g-C$_3$N$_4$, and O-g-C$_3$N$_4$. The enhanced antiviral performance was inferred to be due to improved charge separation by heterojunction formation, resulting in primarily *OH generation under photocatalysis, in turn causing oxidation, defect formation, and rupture of viral capsids [166].

Investigating another type of g-C$_3$N$_4$ heterostructure, Cheng et al. investigated Ag$_3$PO$_4$/g-C$_3$N$_4$ composites and their activities against bacteriophage f2. For these systems, in which the Ag$_3$PO$_4$ nanoparticles were distributed uniformly on the g-C$_3$N$_4$ sheets, photoluminescence results showed that the carrier recombination rate was lower for Ag$_3$PO$_4$/g-C$_3$N$_4$ than for Ag$_3$PO$_4$ and g-C$_3$N$_4$ alone. From a functional perspective, $\log$ 6 inactivation of bacteriophage f2 ($3 \times 10^6$ PFU/mL) was observed within 80 min in the presence of Ag$_3$PO$_4$/g-C$_3$N$_4$, demonstrated from radical scavenger experiments to be due to $h^+$ and *OH [167].

6. Outlook

As outlined in this overview, a range of nanomaterials display photocatalytic properties and generate oxidative stress through •OH formation on exposure to light. Through oxidative degradation of lipid membranes, proteins, DNA/RNA, and other essential components, such photocatalytic inactivation represents a powerful way to combat bacteria and viruses. As exemplified, the range of nanomaterials displaying such photocatalytic effects is wide, and the number of combinations and heterostructures displaying potent antibacterial and antiviral effects seemingly endless. Considering this, as well as the need of novel antibacterial and antiviral therapeutics to combat challenging infections, the importance of photocatalytic nanomaterials in this context is likely to increase (Fig. 10).

From a mechanistic perspective, however, much remains to be elucidated in order to understand selectivity between pathogens and human cells. (This concerns not only the application of such nanomaterials as antimicrobial and antiviral agents, as discussed in the present overview, but also in the application of such nanomaterials for pathogen detection [169].) For example, while some recent studies have shown that presence of anionic and/or polyunsaturated phospholipids results in an increased susceptibility to photocatalytic degradation of lipid membranes, open questions relate to effects of: (i) anionic phospholipid concentration, (ii) nature of the anionic headgroup, and (iii) to what extent anionic phospholipid distribution along the bacterial membrane affect the loci of oxidative destabilization. Similarly, while some studies have demonstrated an increased robustness towards oxidative destabilization of lipid membranes in the presence of cholesterol, key issues remain largely unaddressed in this context, including the influence of: (i) cholesterol content, (ii) raft formation, and (iii) nature of the sterol (e.g., cholesterol vs ergosterol for understanding selectivity towards fungi). Furthermore, while some studies have shown that LPS can be degraded by photocatalytic nanoparticles, the influence of bacterial lipopolysaccharide structure on their susceptibility to photocatalytic degradation is presently unknown, and signifies a need for mechanistic studies of lipopolysaccharides of different length (e.g., “rough” vs “smooth”) and of different charge density and size of

### Bacterial membranes

- Selectivity between pathogens and host cells
- Oxidative susceptibility of different membrane components
- Broader toxicity profile
- Antimicrobial spectrum
- Resistance development
- Approaches for deeper tissue penetration

---

**Key challenges/development steps:**

- Selectivity between pathogens and host cells
- Oxidative susceptibility of different membrane components
- Broader toxicity profile
- Antimicrobial spectrum
- Resistance development
- Approaches for deeper tissue penetration

---

**Fig. 10.** On illumination, a wide range of photocatalytic nanomaterials induce oxidative degradation of membrane components, including phospholipid membranes, bacterial lipopolysaccharides (LPS/LTA), and viral capsid proteins. As a result of this, such nanomaterials display potent antibacterial and antiviral effects. In order to translate current research towards therapeutic applications of such materials, however, a number of key issues need to be further addressed, including selectivity between pathogens and host cells, in vivo toxicity, spectrum width, resistance development, as well as approaches for reaching deeper tissue penetration to allow such nanomaterials to be employed for a wide range of indications. (Elements re-drawn from [35] and [168].)
hydrophobic domains (e.g., LPS vs LTA). Also effects of thickness and cross-linking density of peptidoglycan on differences observed between Gram-positive and Gram-negative bacteria remain largely unexplored from a mechanistic perspective. For the design of novel photocatalytic nanomaterials displaying the selectivity between pathogens and human cells required for their application as therapeutics, all these aspects therefore require further research.

Current translation of academic research on nanoparticles towards novel antimicrobial agents varies between nanoparticles [170,171]. In some fields, such as anti-infectives, AgNP and TiO₂ have been commercially available for years. With regards to therapeutic applications, however, the field is much less mature. In particular, toxicity of photocatalytic nanomaterials is still poorly understood, and the majority of academic studies of such materials involve only basic toxicity assessment on a single cell type. Instead, toxicity assessments in vitro for any therapeutic development of antimicrobial therapeutics need to include several human cell types, using several different assays. This needs to be complemented with toxicity assessment in vivo, including, e.g., effect on inflammation, complement activation, and coagulation, as well as on pharmacokinetics and accumulation-related side effects, all matching the target indication with respect to expected dosage, location, and duration of treatment.

Furthermore, the issue of antimicrobial spectrum width requires much larger attention in order to clarify which bacterial and viral strains that are susceptible to photocatalytic deactivation by a given nanomaterial, and which are not. Studies on spectral width need address also bacterial biofilms, since the latter are frequently formed in challenging infections, and since mass transport limitations make treatment of such infections by of nanoparticles difficult, particularly in vivo. In this context, resistance development also needs much more attention, both for photocatalytic nanoparticles alone and in combination with antibiotics and other therapeutics. With research in the area of antimicrobial nanoparticles as therapeutics being largely motivated by resistance development against conventional antibiotics, the limited attention paid so far to resistance development against antimicrobial nanomaterials is striking [172–174]. Resistance development is a particularly important issue for nanoparticle-based antimicrobials, as these are frequently so cheap that they can be broadly applied also as antiseptic agents, exemplified by the excessive use of AgNPs in a wide range of products. Considering this, research is urgently needed on resistance development against antimicrobial nanomaterials, including mechanisms of resistance development, resistance propagation between different types of nanoparticles, and effects of resistance development on bacteria fitness.

With regards to indications, a fundamental limitation of photocatalytic antimicrobial nanomaterials relates to the need of light to reach the nanomaterials at sufficient intensity in order to generate ROS and trigger oxidative destruction of pathogens. Particularly for UV-light, tissue penetration is very modest, restricting the use of photocatalytic nanomaterials as antimicrobial agents essentially to external surfaces, such as skin/wounds and some mucosal surfaces. For nanomaterials displaying photocatalysis under visible light, penetration depth is larger, and for NIR even larger radiation penetration is observed. Therefore, photocatalytic nanomaterials triggered by visible light or NIR open up more deeply localized infections. Considering this, extending the use of photocatalytic nanomaterials as therapeutics beyond the (albeit important) external surfaces, there is a need for further work on the design of nanomaterials that can be effectively triggered by NIR for versatile therapeutic use. In such a development, the items raised above need to be simultaneously incorporated, i.e., reevaluating considerations of the risk–benefit balance and of health economic consequences ultimately determine whether or not a particular system is suitable for a particular indication. In this context, synergies can likely also be found between antimicrobial photocatalysis and chemodynamic approaches, the latter providing localized ROS generation by chemical means [175,176], for combating severe infections.

Declaration of Competing Interest

The authors have no conflicts of interest to report.

Acknowledgement

This work was financed by the Swedish Research Council (grant number 2016-05157; MM), Independent Research Fund Denmark (grant number 9040-00020B; LPO, MM) and the LEO Foundation Center for Cutaneous Drug Delivery (grant number 2016-11-01; MM).

References

E. Parra-Ortiz and M. Malmsten

Advances in Colloid and Interface Science 209 (2022) 102526

17


[34] Liu P, Duan W, Li X. The damage of outer membrane of Eschericha coli in the presence of TiO$_2$ nanoparticles with combined UV light. Colloid Surf B 2010;78:171-6.


Advances in Colloid and Interface Science 209 (2022) 102526

18

18


[38x104][14597]


[327x240][327x208]interspersed g-C$_3$N$_4$/AgBr-modified g-C$_3$N$_4$ heterostructure for rapid sterilization. J Hazard Mater 2020;379:93.

[327x280][327x208]8 .

[327x288][327x208]8 .

[327x280][327x208]28 .

[327x290]+[327x208]3 .


[327x487]–[327x519]84 .

[327x519]–[327x543]in vitro.

[327x527]–[327x583]86 .


