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MINI-REVIEW

Functionalization of biomolecules on nanoparticles: specialized for antibacterial applications

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Abstract Biological efficiency of existing antimicrobial agents is still inadequate to ensure optimal therapeutic index. Developing biocompatible advanced functional materials with antimicrobial properties could be promising for environmentally benign applications. Nanoparticles and other nanoscale materials are of great interest due to their multiple potential applications in material science, medicine, and industry. Nanomaterials possess well renowned antimicrobial activity against several microorganisms; however, it has some non-specific toxicity. Biofunctionalization of nanomaterials is one such topic to address this issue. Rational selection of therapeutically active biomolecules for design of nanoparticles will certainly increase the biological applicability. The present paper describes the current status of different types of biofunctionalized nanoparticles and their antibacterial applications. Key principles such as strategies involved at bio-/nanointerface, the structural activity relationship, and mechanism of action involved in the antibacterial activity of functionalized nanoparticles are briefly discussed. This knowledge is important from the objective of generation of advanced functional nanomaterials with antimicrobial properties.

Keywords Biomolecules · Nanoparticles · Functionalization · Bio-/nanointerface · Antibacterial application

Introduction

Recently, numerous antimicrobial drugs have been developed to kill or inhibit the growth of pathogenic microbes. However, the therapeutic efficiency of these drugs is still inadequate to attain the optimized therapeutic index (Zhang et al. 2010). The combination of material science and nanomedicine has resulted in the emergence of a new alternative field that involves functionalizing nanostructures with several biologically active materials (Veerapandian and Yun 2009). These advanced materials have unique and unusual physicochemical properties, such as ultra-small sizes, large surface area/mass ratio, chemical reactivity, and high compatible for surface modification. Furthermore, the dimensions of nanomaterials and biomolecules (such as small peptides, proteins, and nucleic acids) are generally similar, which allow these particles to display functions and properties that are similar to normal biomolecules present in cellular systems. Covalent bonding (Veerapandian and Yun 2010), non-covalent bonding (Dietmar et al. 2009), coupling reaction assisted immobilization (Wang et al. 2004), simple coating/deposition (Christopher et al. 2007), Stöber technique (Trewyn et al. 2007; Heather et al. 2007), and reverse micelle and sol–gel techniques (Yang et al. 2004) are widely used methods for biomolecule functionalization of nanostructures, whereas vacuum-UV radiation, radio frequency plasma, and photo-Fenton oxidation (Mazille et al. 2010) have been used to modify nanostructured surfaces for click chemistry. The typical feature common to all functionalizing techniques is the formation of complex moieties between two complementary substances.

Short amphiphilic peptides naturally have antimicrobial and immunomodulatory activities (Zasloff 2002). In mammals, these peptides exhibit rather weak antimicrobial activity under some physiological conditions, and their ability to initiate immune responses through unique mechanisms is more important (Oppenheim and Yang 2005). The cellular prion-related protein is a cell-surface protein that is also abundantly expressed in the human

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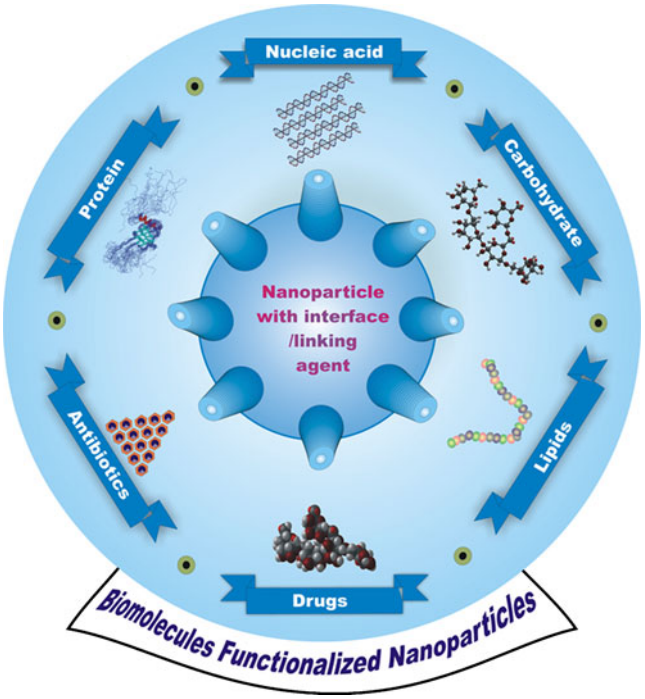
body. This multifunctional prion protein and the exposed cationic and heparin-binding N-terminus both display antibacterial properties (Pasupuleti et al. 2009). Nanostructures that are functionalized with simple saccharide and polysaccharide also display antibacterial properties. For instance, naturally occurring polysaccharide have film-forming ability, can act as chelators, and are polycationic. These molecules also exhibit a wide variety of other attractive biological activities, including antimicrobial activity, disease resistance in plants, and diverse stimulating or inhibiting functions in a number of human cell types (Wang et al. 2006). Next to antimicrobial peptides, lipids such as fatty acids and their derivatives are known to have self-antimicrobial disinfecting activity on the skin surface (Drake et al. 2008). Free fatty acids are secreted from sebaceous glands as sebum triglycerides and consequently released through hydrolyzation by enzymes such as lipases from anaerobic bacterial species (Holland et al. 1981). Numerous free fatty acids have been reported to display antibacterial activity against a range of Gram-positive bacteria but not Gram-negative bacteria (Georgel et al. 2005; Patel et al. 2008). In addition to the biomolecules described above, there are wide varieties of bioactive natural product available that have been used as drugs both commercially and in research. These drugs have been examined in different drug delivery systems for antimicrobial therapy (Zhang et al. 2010). The formation of metal complexes and their specific reaction at sites on long DNA strands provide a route toward the rational development of chemotherapeutic and antimicrobial agents (Niemeyer 2001).

General routes for coupling and organizing biomolecules on nanoparticles (Katz and Willner 2004) for biosensors, nanobiotechnology, and nanoelectronics have already reported (Niemeyer 2001). In addition to that, functionalized gold nanoparticles for pathogenic bacteria (Pissuwan et al. 2010), nanoparticles in antimicrobial material (Weir et al. 2008), and silver nanoparticle-based antimicrobial activities (Virender et al. 2009; Sadhasivam et al. 2010) have also been studied. Although there have been many reports related to the basic guidelines for functionalization and application of specialized nanomaterials are available in the literatures, research on functionalizing nanomaterials with therapeutically active biomolecules (characterized by peptides/proteins, nucleic acid, carbohydrates, lipids, and antibiotics) and their specific antibacterial activity is poor in the literature. Thus, in this review, the current status of biomolecules functionalized nanomaterials with antibacterial property was explored. In addition, basic class of nanomaterials and its general applications, types of functionalization, and antibacterial mechanism of nanoparticles are discussed. Scheme 1 depicts the general types of biomolecule and drugs that have been utilized for nanoparticle functionalization.



Scheme 1 Functionalization of nanoparticles with biomolecules in the presence of interface/linking agents for antibacterial applications

Categories of nanoparticles: functionalization and antibacterial mechanism

To date, a wide variety of nanoparticles and their composites exist already, and diverse top–down or bottom–up approaches for synthesis have been developed. The biomedical application of these nanoparticles may be altered according to size, shape, and surface functionalization. Table 1, describes the different class of nanoparticles and their relevant applications. By properly functionalizing nanoparticles with chemical/biological groups, the physicochemical and biological behavior of the particles can be altered based on their environment, leading to more optimized applications (such as drug delivery). Table 2 displays the general functionalization and biofunctionalization processes used with nanoparticles for different biological and biomedical applications. Understanding the antibacterial mechanism of designed nanoparticles is important for achieving the synergistic effects with biomolecules. Generally, the cell cytotoxicity mechanisms for metal nanoparticles (such as Ag, TiO_2, CeO_2, ZnO, Al_2O_3, and Fe_3O_4) are demonstrated to be reactive oxygen species (ROS) production (Nel et al. 2009). In addition, dissolution and release of toxic cations from nanomaterial surface result in inhibition of respiratory enzymes, ATP production, disruption of membrane integrity, and transport processes that ultimately lead to the death of the bacterial cells (Wen-Ru et al. 2010; Tian et al. 2008). Cytotoxicity mechanism

Table 1 Types of nanoscale materials with significant examples and its general applications (Veerapandian and Yun 2009; Faraji and Wipf 2009; Gaucher et al. 2010)

Material classification	Applications
Inorganic materials	
Noble metals like Au, Ag, Pt, Pd	Photothermal cancer therapy, disease diagnostics, optical imaging, Raman probe, drug delivery, antimicrobial, etc.,
Carbon based materials Carbon nanofibers (CNFs) Multiwalled nanotube (MWNT) Double walled nanotube (DWNT) Sing walled nanotube (SWNT)	Nano-reinforcement, catalytic supports, chemical/biochemical sensing, neural or orthopedic implants, hydrogen storage materials, anode materials in lithium batteries, vaccine delivery system, DNA transporters, NIR photothermal agent by receptor mediated endocytosis, etc.
Semiconducting materials CdSe, CdS, ZnS, TiO_2, PbS, InP, Si/SiO_2	High-resolution and sensitive cellular imaging, long-term in-vivo observation of cell trafficking, in-vitro imaging of pre-labeled cells, tumor targeting, and diagnostics, quantum computation, electronic devices fabrication, etc.
Magnetic materials Fe_3O_4, Co, $CoFe_2O_4$ FePt, CoPt and their composites	Magnetic hyperthermia, magnetic immunoassay, biomedicine, magnetic resonance imaging (MRI), magnetic particle imaging, environmental remediation, catalysis, etc.
Lanthanide materials Gd_2O_3, Eu_2O_3	MRI contrast agent, contrast enhancing agent, luminescent bioassay, biolabels for multi-label time-resolved fluoro-immunoassay
Polymer materials	
Simple polymeric nanoparticles such as gelatin, PGA, PBCA, PMMA, PACA, block copolymers such as PEG-*b*-PLGA and PEG-*b*-PDLLA, polymeric micelles such as PLA, PCL, PAA, PAsp, PGlu, PLys, PHis and dendrimers (PAMAM)	Drug delivery systems include parenteral, targeted therapy to solid tumors, solubilization, stabilization and improve the biodistribution of a wide range of therapeutic agents
Polysaccharide materials	
Alginate (algal origin), pectin, guar gum (plant origin), dextran, xanthan gum (microbial origin) and chitosan, chondroitin (animal origin)	Used as a surface modifier and reducing agent for synthesis of several nanoparticles, drug delivery, gene delivery, etc.
Lipid materials	
Liposomes Lipopolyplexes/polyplexes Solid lipid nanoparticles	Non-viral carrier for genes, intracellular delivery of hydrophilic molecules (nucleic acids, messenger RNA, peptides and proteins)
Virus capsids and related cage-like proteins	
Cowpea chlorotic mottle virus (CCMV), vault nanocapsules, canine parvovirus (CPV), adeno-associated virus (AAV), heat shock protein, hepatitis B cores, ferritin, MS2 bacteriophages, and M13 bacteriophages	Biomaterials, vaccines, diagnostic imaging, molecular electronic materials, template for synthesis of hard (inorganic) or soft (organic) materials, tumor targeting agents, etc.

PGA Poly(glycolic acid), *PBCA* poly(butyl)cyanoacrylate, *PMMA* poly(methylmethacyrlate), *PACA* poly(alkylcyanoacrylate), *PEG-b-PLGA* poly (ethylene glycol)-block-poly(lactic-co-glycolic acid), *PEG-b-PDLLA* poly(ethylene glycol)-block-poly(D,L-lactide), *PLA* poly(lactic acid), *PCL* poly (caprolactone), *PAA* poly (amino acid), *PAsp* poly(aspartic acid), *PGlu* poly(glutamic acid), *PLys* poly(L-lysine), *PHis* poly(histidine), *PAMAM* polyamidoamine

for carbon-based nanomaterials, such as single-walled carbon nanotubes (SWCNTs), multi-walled carbon nanotubes (MWCNTs), fullerenes, and graphene are still at infant level and subject to criticism. Preliminary reports mainly focusing on mammalian cells proposed three principal cytotoxic mechanisms (for SWCNTs), such as oxidative stress, metal toxicity, and physical piercing, reviewed elsewhere (Seoktae et al. 2008). On the other hand, MWCNTs exhibits antibacterial activity mainly due to cell membrane damage by direct contact (Seoktae et al. 2008). Cationic nanospheres and dendrimers are reported to have proton sponge effect, leading to lysosomal damage-derived induction of cytotoxicity (Nel et al. 2009). Quantum dots (QDs) are nanoscale crystalline clusters prepared from semiconducting materials. Recently, antimicrobial mechanism of QDs (CdTe) was investigated against *E. coli*, which involves both the surface binding and a ROS-dependent pathway. Furthermore, CdTe QDs also down-regulated the anti-oxidative genes, resulting in low level translations of anti-oxidative enzymes. This effect influenced the oxidant/anti-oxidant balance and accumulates ROS in bacteria, leading to biocidal effects (Zhisong et al. 2008). From the above literatures, it is possible to understand the fundamental cell cytotoxicity mechanism of nanomaterials.

Table 2 Common types of functionalization and biofunctionalization of nanoparticles (Thanha and Green 2010; Subbiah et al. 2010)

Class of functionalization and biofunctionalization	Uses
Ionic stabilization	Provides stability and dispersion of inorganic metal nanoparticles
Steric stabilization Polymeric ligands Small molecular ligands	Physical barrier to prevent aggregation of several nanoparticles include, metal, semiconducting and magnetic nanoparticles
Ligand exchange Oxygen-based ligands Neutral and anionic ligands Nitrogen-based ligands Neutral and cationic ligands Phosphorous-based ligands Sulfur-based ligands Neutral and anionic ligands	Increased binding strength via formation of self assembled monolayer's and their derivatives on the surface of nanoparticles and provides high degree of protection
Ligand addition Core–shell modification Hydrophobic interaction	Used to modify the external surface of the nanoparticle-ligand shell layer without removing the preexisting ligands and further protect the core material
Coupling strategies for biofunctionalization	
Carbodiimide coupling	Used to covalently link –COOH group (on the surface of nanoparticles) to amines (in biological species) via formation of a "zero length" amide bond
Maleimide coupling	Conjugation of biomolecules such as DNA, herceptin and proteins to nanoparticles
Click chemistry	One-step click process provides number of nanoparticle-ligand bond formation (example, Cu-mediated alkyne-azide cycloaddition)
Disulfide bridges	Reversible chemical coupling of nanoparticles
Ionic coupling	Coupling of nanoparticles and oppositely charged biological/polymer species
Specific biorecognition interaction	For binding the proteins, antibody and nucleic acids, to the nanoparticle *via* an active site irrelevant to the biomedical application

Biomolecules on nanoparticles: bio/nanointerface strategy

Optimizing the interface between biomolecules and nanostructured materials is currently a promising path of research and development. Self-assembled colloidal nanoparticles and biomolecules can adopt several different nanostructures, including nanospheres, nanoshells, core–shell composites, nanoneedles, and nanorods. Understanding the basic phenomena of biological interactions using nanoscale materials as a probe molecule will help unravel the physicochemical and biological properties of materials. Several key biological properties need to be controlled while working on bio-/nanointerfaces especially for biological applications, including adverse immune responses and biocompatibility. The in vitro and in vivo targeting and extraction of cancer cells using magnetic nanoparticles conjugated with peptides (YSA: YSAYPDSVPMMS) was demonstrated (Kenneth et al. 2008). In this work, the researchers used polygalacturonic acid as a biocompatible interface agent and a functional group that specifically targeted ovarian cancer cells expressing EphA2 tyrosine kinases receptors. Biocompatibility can also be controlled by fabricating stable nanomaterials. However, the stabilizing moieties currently used in the nanomaterial fabrication protocols are still not well-established (Charusheela 2010). Recently, Charusheela examined the in vitro and in vivo stabilization of nanomaterials using biomolecules. In that work, different kinds of biological molecules were used during the biosynthesis of nanoparticles, and the mechanism of stabilization was examined. That study further reviewed the role of biomolecules such as proteins, peptides and phytochelatins in the in vitro and in vivo stabilization of nanomaterials (Charusheela 2010). Examples of nanomaterials and the corresponding interface/linking agents utilized for the functionalization of active antimicrobial biomolecules are listed in Table 3. Apart from these interface agents, other active interface groups such as thiol/dithiol (octanethiol, dodecanethiol, 1,6-hexanedithiol, and 2,8-octanedithiol), aminothiol (2-aminothiol), silanes (mercaptosilane), phosphine (triphenyl phosphine), and succinimidyl 4-(*N*-maleimidomethyl)-cyclohexane-1-carboxylate have been the most frequently used compounds for biofunctionalization. The key principle involved in the above bio-/nanointerface strategy is the interfacial activity. Interfacial activity is the potentiality of an interacting

Table 3 Examples of biomolecule functionalized nanoparticles in presence of interface/linking agent and its antibacterial application

Nanomaterial	Interface/linking agent	Biomolecule as functional entity	Application	References
Starch-AuNPs	Thiotic acid	Bombesin, Annexin V	Cellular interaction and apoptosis	(Swapna 2008)
AuNPs-mPEG-SH	Succinimidyl 6-[3′-(2-pyridyldithio)-propionamido] hexanoate	Anti-neurofilament rabbit IgG	Bioconjugation	(Hongwei and Jason 2005)
AuNPS-NH_2	EDC	Anti-PA3	Antibacterial (*P. aeruginosa*)	(Norman et al. 2008)
AuNPs	–	Vancomycin-cystamide	Polyvalent inhibitor of vancomycin resistant enterococcus	(Gu et al. 2003)
Fe_3O_4@AuNPs	–	Vancomycin-cystamide	Magnetic, NIR absorption and antibacterial	(Huang et al. 2009)
AuNPs	–	*Rhizopus oryzae*	Microbicide, water purification	(Das et al. 2009)
AuNPs	–	Gentamycin	Bactericide (*E. coli* K12)	(Burygin et al. 2009)
AgNPs	–	Gelatin	Bactericide (*P. aeruginosa and S. aureus*)	(Xu and Zhou 2008)
AgNPs	PEO	Chitosan fiber	Bacteriostatic agent (*E. coli*)	(An et al. 2009)
AgNPs	Polyimide film	Dopamine	Antibacterial (*E. coli*)	(Liao et al. 2010)
MWNT-COOH	EDC/NHS	Lysostaphin and ALE1	Bactericide (*S. aureus* and *S. epidermidis*)	(Pangule et al. 2010)
SiO_2/TiO_2	–	Lysozyme	Bacteriolytic acitivity (*M. lysodeikticus*)	(Luckarift et al. 2006)
Ag loaded SiO_2	3-Aminopropyl triethoxysilane (KH550)	Wool fiber	Antibacterial wool fiber (*E. coli* and *S. aureus*)	(Wang et al. 2007)

EDC 1-Ethyl-3-(3-dimethylaminopropyl) carbodiimide, *PEO* polyethylene oxide, *NHS* *N*-hydroxysuccinimide, (–) not available

molecule to adhere to a surface and partition onto one surface and other interface through packing or altering the organization of molecules (Rathinakumar et al. 2009). Recently, a report reviewed the various biophysicochemical properties of the bio-/nanointerface between biomolecular systems and nanomaterials. In this work, they examined the different characteristics of nanoparticles, such as size, shape, surface charge, roughness crystallinity, and solubility. Furthermore, they investigated the important interaction forces like receptor–ligand interaction, membrane wrapping, biomolecular forces, and energy transfer mechanism. Sorption of steric molecule, electrostatic and electrosteric interaction, electrical double-layer formation, zeta potential, and isoelectric point were also found to be important factors at the interface layer (Nel et al. 2009). A general schematic representation of the interaction between functionalized nanoparticles and bacterial surface is shown in Fig. 1. As shown in this figure, Gram-positive bacteria (Fig. 1b) have complex layers of peptidoglycan that contains teichoic and lipoteichoic acid, which have a strong negative charge (Kawahara et al. 2000). Nanoparticles with an increased surface area and release of ions cause physiological changes in the bacteria and disrupt the metabolic activity of bacteria resulting in cell death (Feng et al. 2000; Morones et al. 2005). The complex mechanism involved in this process depends on the biophysicochemical properties of the nanoparticles (Nel et al. 2009).

Peptide and proteins functionalized nanoparticles as antibacterial agent

Peptides, especially cationic antimicrobial peptides, have been shown to specifically act against several multi-drug-resistant microbes (Hancock and Sahl 2006). The antimicrobial properties of several peptides are based on different factors such as the formation of α-helical (Oren et al. 2002) or β-sheet like tubular (Lopez et al. 2001) structures or α-helical bundles, which result from the interaction with the negatively charged cell surface and self-association in the solution state (Avrahami and Shai 2002). After engaging additional peptide monomers, this process results in the disintegration of the cell membrane. The transcriptional activator (TAT; YGRKKRRQRRR) peptide is the viral gene encoded in human immunodeficiency virus type-1 (HIV-1) (Vives et al. 1997). Chemical functionalization of TAT on other biomolecules such as proteins (Fawell et al. 1994), small interfering RNA (siRNA) (Turner et al. 2007), and nanoscale drug carriers like liposomes (Pappalardo et al. 2009) results in enhanced cellular uptake of proteins and genes. It has also been reported that after coupling TAT with proteins of low to high molecular weight (Schwarze et al. 1999), quantum dots and other

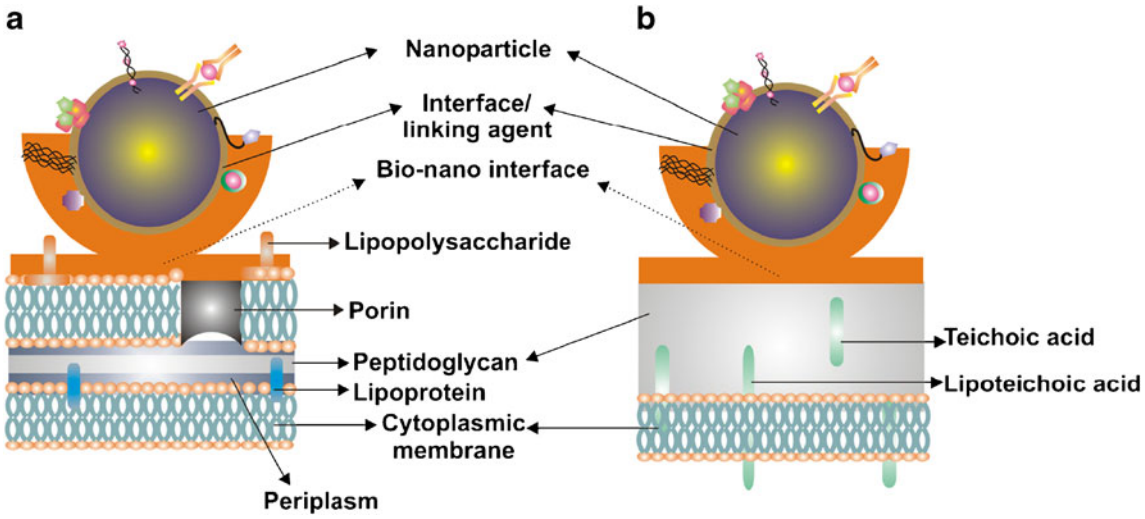


Fig. 1 Illustration of the bio-/nanointerface between functionalized nanoparticles and **a** Gram-negative bacteria and **b** Gram-positive bacteria (the unique structures over the nanoparticles represent the different active biomolecules as functional moieties for antibacterial purpose)

nanoscale materials such as polymeric micelles could cross the blood–brain barrier (BBB). This is a highly important attribute to drug delivery because most drugs fail to cross the BBB, which results in a low therapeutic value of the drug. Recent reports have suggested that the self-assembled core–shell nanoparticles formed by self-assembly of amphiphilic peptide have potential antimicrobial activity against a wide range of bacteria, yeasts, and fungi (Lihong et al. 2009). The therapeutical index of this nanomaterial against *Staphylococcus aureus* infection in mice was shown to be more potent than the unassembled peptide moiety. This amphiphilic peptide (CG_3R_6 TAT) contained TAT as the hydrophilic cell-penetrating peptide, six arginine residues (R_6) to increase the membrane translocation, and a hydrophobic block composed of cholesterol to improve membrane permeability. In addition to these components, a spacer consisting of three glycine residues (G_3) was built in between to separate the two blocks, which led to the formation of core–shell nanoparticles (micelles). The low density positive charge and peptide mass were key factors controlling the enhanced antimicrobial activity. In addition, functionalization with TAT further improved the BBB crossing ability, which is vital for the treatment of brain infection (Lihong et al. 2009). A solid phase method was used to fabricate the G_3R_6TAT, and CG_3R_6TAT was synthesized by grafting cholesteryl chloroformate onto the N-terminus of the G_3R_6TAT (Lihong et al. 2009). On the other hand, much attention has been given to the conjugation of proteins to polymer nanostructures especially for biomedical applications (Rohan and Alexey 2008). Such structures are easy to fabricate with controlled monodispersity, which also overcomes issues associated with optimizing immobilization, minimizing diffusion limitations, maximizing surface area per unit mass, and increasing the enzyme loading ability (Jia et al. 2003). A previous study examined covalent attachment of hen egg lysozyme to two types of polystyrene latex nanoparticles for directed targeting (Rohan and Alexey 2008). In that study, the antibacterial activity against a Gram-positive *Micrococcus lysodeikiticus* by conjugating lysozymes to positively charged nanoparticles was approximately twice as large as that of the free enzyme. Similarly, the antimicrobial efficacy of the lysozyme was increased by fabricating immunonanoparticles, i.e., nanoparticles functionalized with pathogen specific antibodies, which may serve as an antimicrobial cargo for enriching the stability and activity of antimicrobial in foods. Carboxyl group functionalized polystyrene nanoparticles were conjugated to anti-*Listeria monocytogenes* through a covalent interaction. In this study, the efficiency of lysozyme-carrying immunonanoparticles was higher than the simple lysozyme in regards to inactivating *L. monocytogenes* in nutrient broth (Yang et al. 2007). The combined results of these studies clearly demonstrated that functionalization of nanoparticles with peptides and proteins can significantly enhance the antibacterial function of biomolecules.

Carbohydrate-functionalized nanoparticles as antibacterial agent

Metastasis, inflammation, and infection are the characteristic feature of carbohydrates in a broad spectrum of physiological and pathological processes. All of these processes involve carbohydrate–protein as well as carbohydrate–carbohydrate interactions (Rojo et al. 2004). Recently, we reported that active functionalization of the

naturally occurring amino sugar, glucosamine, on silver nanoparticles (GlcN-AgNPs size, 30±5 nm) displayed higher antibacterial activity than simple silver nanoparticles (AgNPs size, 20±2 nm) against 8 g negative and 8 g positive bacteria (Veerapandian et al. 2010). The minimum inhibitory concentration results indicated that *Klebsiella pneumoniae* (ATCC 700603) and *Bacillus cereus* isolates were more highly inhibited in the presence of GlcN-AgNPs than AgNPs. The possible mechanism of action of GlcN-AgNPs was attributed to the surface functionalization of glucosamine, which helped the glyco-nanoparticles penetrate into the bacterial cell. This increased activity was also due to the larger surface area for contact and interaction with the cell surface and/or a unique activity due to the formation of the secondary amide bond (Veerapandian et al. 2010). Figure 2 shows representative FE-SEM images of the AgNPs and GlcN-AgNPs. Similarly, the antibacterial activity of natural polysaccharide chitosan has been extensively studied because of their excellent biocompatibility, biodegradability, and metal complexation (Mao et al. 2001). Previous reports have suggested that the antibacterial activity of this biomolecule was prominently enhanced when attached to nanoparticles through metal ions such as Cu^{2+} and Zn^{2+}. Furthermore, this increased activity was found to be directly proportional to the zeta potential (Wen-Li et al. 2009). In another report, a crosslinked chitosan (CCTS) coated Ag-loaded nano SiO_2 composite (CCTS-SLS) was reported to have a high antibacterial activity against *Escherichia coli* and *S. aureus* due to the coordinated action of CCTS and SLS (Niu et al. 2009). Aminoglycosides, which are amino-modified sugars, have several well-reported antibiotic properties. However, in general, the development of bacterial resistance is common to all antibiotics. A previous study examined the antibacterial efficiency of gold nanoparticles that had been functionalized with aminoglycosides (Nirmala Grace and Pandian 2007). In this study, the activity of aminoglycosidic antibiotics, such as streptomycin, gentamycin, and neomycin, were protected when immobilized on gold nanoparticles.

Lipids functionalized nanoparticles as antibacterial agent

Antimicrobial lipids are part of the innate immune system (Germain 2001). The innate immune system plays several important roles, including the first line of defense against microbial organisms, controlling the activation of adaptive immunity, and determining the type of effect or response to certain pathogens (Medzhitov and Janeway 2000). There are some important antimicrobial lipids found in human skin cells, such as lauric acid, sphingosine, sapienic acid, dihydrosphingosine, and 6-hydroxysphingosine. These endogenous antimicrobial lipids have natural protection against common potential pathogens (Drake et al. 2008). In 1988, the role of lipids in enhancing the antibacterial activity of benzoyl peroxide against *Propionibacterium acnes* was reported. Recent reports have suggested that the antimicrobial lauric acid has antibiotic properties against acne vulgaris (Christopher Decker et al. 1989). Generally, long chained fatty acid molecules, such as stearic acid and octadecyl amine, are used as stabilizers, which passivates the nanoparticles and prevents aggregation under normal solution conditions (Yamamoto and Nakamoto 2003). Oleic acid is a mono-unsaturated omega-9 fatty acid that has been used in the synthesis of silver nanoparticles. That oleic acid stabilized silver nanoparticles exhibited high antibacterial activity against both Gram-negative *E. coli* and Gram-positive *S. aureus* bacteria (Le et al. 2010). Significantly, the antibacterial activity of AgNPs exhibited a quicker response against *E. coli* than *S. aureus*. These results are shown in Fig. 3, which shows the difference in the thickness of peptidoglycon layer in the bacterial strains. At different magnifications and sections, many AgNPs were located around the cell membrane of both strains as well inside the cells. The increased permeability of the nanoparticles into bacterial cells led to a loss of cellular transport through the plasma membrane, which ultimately resulted in cell death (Le et al. 2010; Yang et al. 2009).

Green chemical synthesis of materials is a promising way to acquire environmentally benign materials. Vegetable oil-

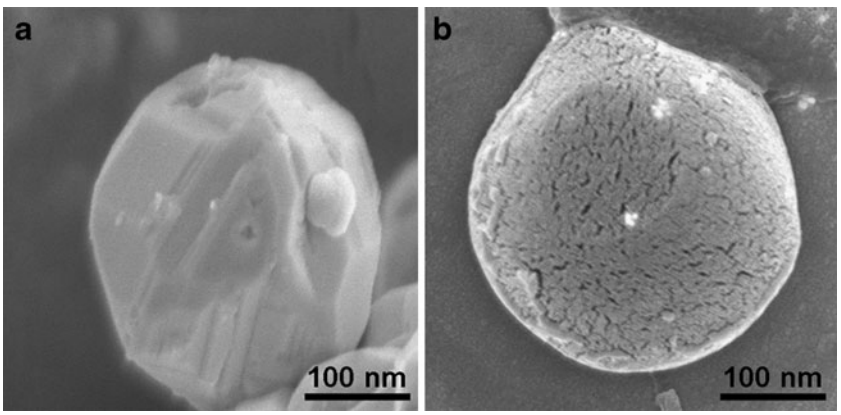


Fig. 2 Depicts the FE-SEM surface morphology of AgNPs (**a**) and GlcN-AgNPs (**b**). Successful surface functionalization of glucosamine is indicated by the smooth spherical morphology over the nanoparticle (**b**)

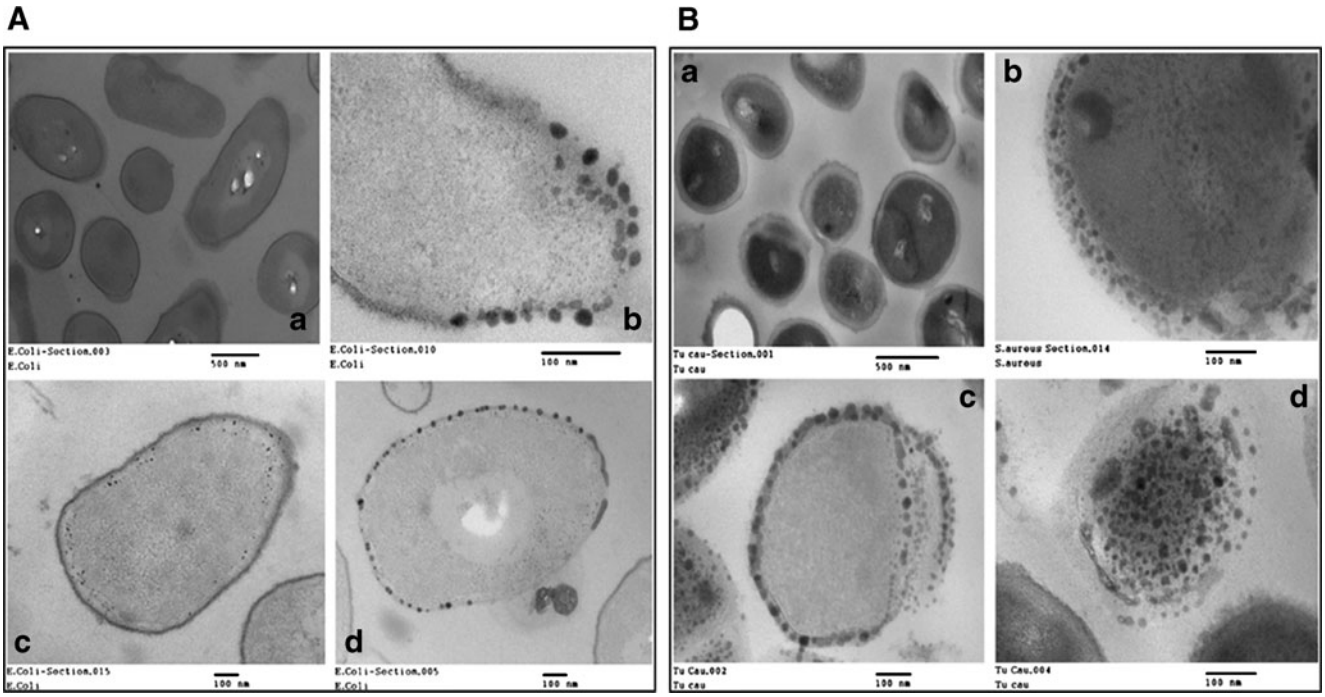


Fig. 3 TEM images shows the interaction stages of oleic acid stabilized AgNPs against **a** *E. coli* and **b** *S. aureus* after *a* 0 min, *b* 30 min, *c* 1 h and *d* 2 h, respectively (reproduced with permission from Le et al. 2010, copyright 2010, Elsevier)

based antimicrobial paints composed of fatty acids and silver nanoparticles have been synthesized in a single step. The mechanism of this reaction is the general free radical exchange that occurs in the oxidative drying process in oils (Ashavani et al. 2008). Appropriate selection of organometallic salts plays a key role in the solubility of the nanoparticles in oil medium. In order to achieve this reaction, researchers have utilized silver benzoate as a precursor salt and an alkyd resin that consists of fatty acids (stearic acid, oleic acid, linoleic acid, and linoleinic acid in drying oils). Under these conditions, the in situ generated aldehydes can be used as protecting agents for the in situ synthesis of nanoparticles. Autoxidation of drying oils was used to reduce metal salts to metal nanoparticles (Sondi and Salopek-Sondi 2004). To examine the antibacterial properties of these particles, glass slides were coated with AgNPs-embedded paint and then sprayed with airborne bacteria (5×10^6 cells/ml for *S. aureus* and to 5×10^7 cells/ml for *E. coli.*). However, the mechanism of action is still not known. It has been explained based on the interaction of AgNPs with the membranes of bacteria results in physiological changes, which eventually lead to the death of bacterial cell (Ashavani et al. 2008; Sudhir 1998).

Antibiotics functionalized nanoparticles as antibacterial agent

The particular reason for including antibiotics in this paper is because of its high affinity toward certain biomolecules. Different antibiotics target various bacteria in a unique manner; thus, studying the particular interaction between broad spectrum antibiotics and harmful pathogens is highly important to rational drug design. Liposomes and nanoparticles loaded with antibiotics have been reported to have increased drug concentration at infected sites with reduced drug toxicity (Huguette et al. 2000). Intracellular infections are particularly difficult to wipe out because pathogenic bacteria can evade cell death using several ingenious mechanisms include inhibition of phagosome–lysosome fusion, resistance to lysosomal enzymes, oxygenated components, and defensins of host macrophages. Thus, facultative intracellular bacterial pathogens present a major problem (Tulkens 1991). The pharmacokinetic and pharmacodynamic properties of antibiotics play a vital role in dictating intracellular activity including entry, retention, subcellular distribution, and expression of activity in the infected system (Barza 1994). The in vitro release rates of antibiotic-loaded nanoparticles have been reported to be low in ester-free medium and high in the presence of carboxyesterase. Colloidal carriers are degraded in endosomes through the process of endocytosis by phagocytic cells with the help of lysosomal esterases (Guise et al. 1987). During the formulation of colloidal nanoparticles, long side chained monomers are generally used to increase the drug entrapment efficiency. Ciprofloxacin (CIP) encapsulated colloidal poly (DL-lactide-*co*-glycolide) (PLGA) nanoparticles showed a relatively lower antibacterial activity compared to free CIP due to the sustained release

characteristics of the encapsulated system. However, the CIP-PLGA nanoparticles effectively inhibited the growth of bacteria, whereas free CIP did not alter the growth of bacteria. The multiple emulsion solvent evaporation method was used to formulate the CIP-PLGA nanoparticles (Jeong et al. 2008). In another study, the antibacterial properties of antibiotics, such as ampicillin, kanamycin, erythromycin, and chloramphenicol, were reported to be increased when combined with AgNPs. The highest enhancing effect was obtained from amphicillin-AgNPs against test strains. The mode of action was based on the van der Waals interaction and other weak bonds, which led to interactions between amphicillin-AgNPs and the bacterial cell wall. These interactions resulted in cell wall lysis and penetration of nanoparticles into the bacterium. Furthermore, the amphicillin complex with nanosilver reacted with DNA and prevented DNA unwinding, which ultimately resulted in bacterial cell death (see Fig. 4) (Amanulla et al. 2010; Batarseh 2004). Similarly, a synergistic antibacterial effect against *E. coli* was obtained when β-lactam antibiotic (amoxicillin) was combined with AgNPs (Ping et al. 2005). This type of research would be invaluable in establishing novel-conjugated antimicrobial agents.

Functionalization of nucleic acids on nanoparticles

Surface chemistry plays a vital role in the conjugation of biomolecule such as nucleic acids to nanoparticles. Gene therapy is currently being examined to treat and control diseases with the help of nucleic acids (Felnerova et al. 2004). Viral and non-viral vectors are generally used to carry this type of therapy into cells within the body to rectify defective genes and alter other biological functions (Luo and Saltzman 2000). Utilization of viruses as a vehicle for gene therapy is now well-established (Yeh and Perricaudet 1997); however, viral vectors have serious limitations such as undesired cytotoxicity, stimulation of an immune response, lack of targeting specific cell types, low DNA carrying ability, lack of ability to infect non-dividing cells, and other traditional issues like production and packaging (Luo and Saltzman 2000; Zhang and Godbey 2006). On the other hand, non-viral gene delivery systems still struggle from low transfection efficiency due to the lack of a controlled process at the nanoscale. These include stability, site specificity, ability to cross the cell membrane, protection of nucleic acid from nuclease degradation, and release of the functional nucleic acid in

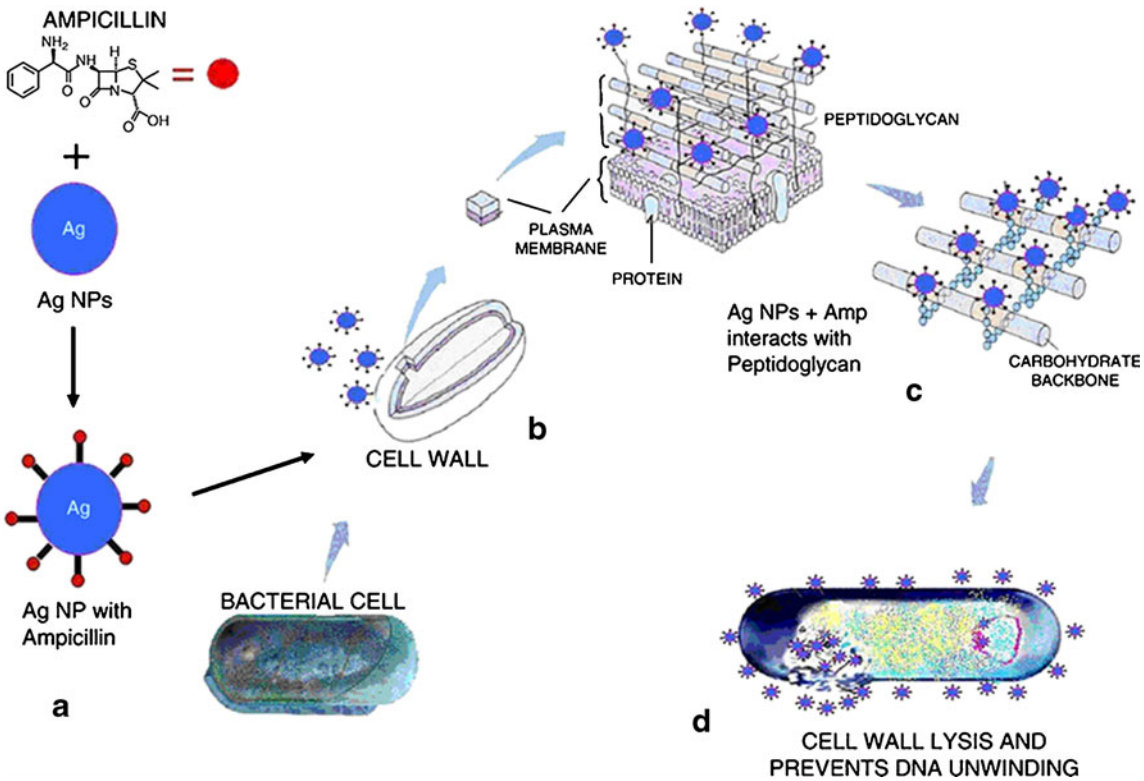


Fig. 4 Synergistic activity of AgNPs with ampicillin (Amp) against bacteria. *A* Formation of core silver nanoparticles with ampicillin. *B* Interaction of AgNPs-Amp complexes with the cell wall of the bacteria. *C* AgNPs-Amp complex inhibits the formation of crosslinks in the peptidoglycan layer (which provides rigidity to the cell wall), leading to cell wall lysis. *D* AgNPs-Amp complex prevents DNA unwinding (reproduced with permission from Amanulla et al. (2010), copyright 2010, Elsevier)

the nucleus (Wiethoff and Middaugh 2003). In the past two decades, nanomaterials, especially hetero-architecture containing nanoparticles, have been applied in biomedical field. Super paramagnetic nanoparticles have been utilized in cell dissociation, protein purification, and genome purification (Merel et al. 1996). Particularly, in bioseparation technologies, simple magnetic fields are used to control the process. Functionalized magnetic nanoparticles are composed of two parts, the core and shell, which provide the compatibility, controlled size, distribution, and sufficient nitrile functional group for coupling or as a surface modification (Lundeberg and Larsen 1995). Furthermore, with regard to biological vector applications, complexation of nanoparticles and DNA could overcome the traditional issues of non-viral carrier. The nanoparticle could also be engineered to specifically deliver the gene of interest to the target tissue (Scherer et al. 2002). For instance, a FePt/ZnS core–shell nanostructure was conjugated with different DNA sequences for target based drug therapy (Ho and Sheng 2010). Gold nanoparticles have been reported to protect DNA from enzymatic degradation and regulation of DNA transcription of T7 RNA polymerase (McIntosh et al. 2001). In another report, gold nanoparticles were complexed with plasmid DNA containing a luciferase gene (Niidome et al. 2004), and using this system, the genes were delivered to HeLa cells in about 3 h. Rod-shaped gold nanostructures also have the potential to deliver nucleic acids to cells or tissues (Bonoiu et al. 2009). In this regard, electrostatic binding was utilized to conjugate siRNA/gold nanorods and the uptake of the conjugates inside the dopaminergic neuronal cells. In addition to the above references, fusion of phototherapy and conventional gene delivery offers synergistic possibilities in gene delivery into cells. Strong and tunable surface plasmon absorption in the nNear-infrared (NIR) range offers gold nanorods as suitable nanomaterial for gene therapy. A report based on the remote control of green fluorescence protein (EGFP) expression in HeLa cells using gold nanorods excited with NIR irradiation was studied. Classical thiolated moiety containing EGFP DNA was used to link Au nanorods through Au–S bonds (Chen et al. 2006). The proposed mechanism was related to the shape-mediated release of DNA from the gold nanorod-EGFP DNA conjugates. These results demonstrated the potential of utilizing novel nanoparticles (such as metal gold and core–shell composite) for the functionalization and delivery of nucleic acids to treat and eliminate harmful viral and bacterial infections.

Summary and outlook

This minireview has summarized recent research works in the biomolecule functionalized nanoparticles for antimicrobial applications. Biomolecules such as proteins/peptides, lipids, carbohydrates, and nucleic acids have huge potential for the antimicrobial application. Using proper functionalization procedures, it is possible to design a stable and highly active biomolecule–nanoparticle hybrid system for many biological applications, such as antimicrobial agents. Physicochemical properties of nanoparticles, interface/linking agents and selective biomolecules play a vital role in dictating the antimicrobial activity. The overall antimicrobial mechanisms from nanomaterials are hypothesized mainly due to ROS production and impacts on cellular membrane integrity and metabolic activity. Understanding the interactions of functionalized nanomaterials and microorganisms/biosystems have just begun; there is still much to unravel the exact mechanism of action. It is expected that a better understanding of the mechanism of functionalized nanomaterials, together with appropriate biointerface phenomena, will permit us to create more standard design features for development of advanced functional materials with desired qualities. We hope that future research will allow us to synchronously select biocompatible/biomolecules as suitable surface modifying or functional ligands for the development of novel therapeutics and other bionanotechnology. The possible interactions between the different shape-controlled functional nanomaterials and biosystems could also be a future research topic.

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