Biological and Pharmaceutical Applications of Nanomaterials

Edited by

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Preface

This book represents recent developments and research activities in the field of nanomaterials with particular focus on biological and pharmaceutical applications.

The book is divided into four sections, each comprising chapters with a common theme. Section I contains seven chapters dealing with nanomaterials for drug delivery. Topics covered in Section I include stimuli-responsive nanostructured silica matrixes, gold nanoparticles, and liposomes for targeting drug delivery applications and dental applications. In addition, material on nanocarriers and nanoparticles as cancer therapeutics and as peptide therapeutics are covered in this section. Section II consists of two chapters dedicated to antimicrobial nanomaterials. Section II covers topics on the influence of surface characteristics on microbial adhesion and summarizes recent advances in antimicrobial nanostructured polymers for medical applications. Section III contains five chapters dealing with nanomaterials in biosensors, and Section IV consists of a single chapter on safety of nanomaterials. Section III covers recent advances in nanodiagnostic techniques for infectious agents, chromogenic biosensors for pathogen detection and electrochemical biosensors for detecting DNA damage and genotoxicity, and molecular imaging with quantum dots including surface modifications by polymers for biosensing applications.

The authors who contributed to this book are very experienced researchers with years of experience in industry and academia. All of the book contributors are experts in their field with considerable experience in researching, developing, and applying the proposed techniques. We sincerely hope that the information in this book will be a valuable resource for clinicians, microbiologists, cell biologists, pharmacists, chemists, and material scientists. This fascinating and comprehensive book will reinforce the multidisciplinary nature of the nanomaterial field.

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Editor

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5 Nanoparticles
A Promise for Host Defense Peptide Therapeutics

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CONTENTS

ABSTRACT

Antibiotic resistance developed by bacterial and fungal pathogens is one of the current major health problems in the world; hence, the development of newer antimicrobial therapies based on novel antimicrobial molecules that diminish this resistance is urgently required. Antimicrobial peptides or, in a wider concept, host defense peptides (HDPs), a diverse set of peptides that are evolutionarily conserved to combat or enhance immunity to infections in all forms of life, could be a reassuring and complementary solution to this health emergency. Many antimicrobial peptides could combine antimicrobial activity with immunomodulatory and anti-inflammatory activities. Although bacteria and fungi have resistance mechanisms against these peptides, their multifunctionality can evade such resistance. HDPs exhibit a broad spectrum of activity against a wide range of microorganisms. Different mechanisms of action have been proposed for these molecules, which indicate that many of them could have more than one antimicrobial target at the cellular level. Many of

them interact with plasma membrane (pore formation, physical and functional disorganization, or simply transit to localize intracellular targets). One of the main difficulties for the utilization of HDPs for microbial control is peptide inactivation by proteinases, which is a real resistance mechanism shared by multiple human pathogens. Also, inactivation in the presence of salts, serum, or microbial components is an additional cause of no *in vivo* activity. The preventive action of HDPs is controversial not only for its effectiveness itself but also from a cost–benefit point of view. The potential immunomodulatory effect of HDP is promising. The combination of new peptides with novel delivery techniques is another approach that could become effective for such peptides. Despite the success in preclinical models, the clinical results of these molecules have not been good enough to approve them for medical use. Alternatives to increase the stability, efficacy, and biodistribution of HDPs are required. Different nanosystems have been demonstrated to develop medical applications. Nanoparticles (1–100 nm) of different materials are characterized by large surface-to-volume ratio, with a large fraction of their atoms located at the surface with unsaturated coordination bonds. Nanoparticles basically are obtained by bottom-up procedures and by top-down routes. They can be functionalized by the incorporation, through acid–base reactions or coordination interactions, of molecular species to allow their conjugation to biomolecules or to provide functional properties. The antimicrobial activity of different types of nanoparticles has been demonstrated when metals exhibit antibacterial properties in their bulk. The antimicrobial effect of these metals increases at nanoscale dimensions. Conjugation of HDPs with nanoparticles could increase the antimicrobial activity of the combined parts. Nanoparticles could be a perfect carrier for HDPs because of their multifunctional activities.

Keywords: Nanoparticles, Antibiotics, Antimicrobial peptides, Host defense, Bacterial infection

5.1 INTRODUCTION

Nowadays, novel infectious diseases have emerged, many of which are responsible for life-threatening disorders (Snell 2003). A lack of new antibiotics for treatment of illnesses, combined with the appearance of multi–drug-resistant strains, has generated the imperative requirement for innovative strategies in the development of newer antimicrobial therapies (Arias and Murray 2009). Host defense peptides (HDPs) are an essential first line of defense against pathogenic infection, showing strictly correlated immunomodulatory and antimicrobial properties. They constitute a novel strategy for the development of antimicrobial therapies (Afacan et al. 2012). Since they mainly act over microbial membranes or host immune cells, HDPs have shown a wide spectrum of activity that included bacteria, virus, and fungi with an ability to cause infectious diseases (Mulder et al. 2013; Theberge et al. 2013). Furthermore, such compounds have also been the focus of attention because of their low risk of triggering microbial resistance owing to their multiple mechanism of action and,

therefore, are also investigated as a novel class of antibiotics, additives, and vaccine adjuvants (Zhang and Sunkara 2014). Cathelicidins and β-defensins are among the most well-known classes of HDPs, which are commonly found in many different animal species (Silva et al. 2011).

Unfortunately, the preclinical success achieved with these molecules has not been translated to the clinic yet (Eckert 2011). The instability, efficacy, and biodistribution of HDPs in clinical trials have been the major drawbacks (Brogden and Brogden 2011). Development of nanoparticles for entrapment and delivery of HDPs could represent an alternative to bypass the abovementioned clinic obstacles (Brandelli 2012). Here, we provide an overview and discuss the potential application of nanoparticles conjugated to HDPs in overcoming infectious diseases.

5.2 HOST DEFENSE PEPTIDES

HDPs, formerly designed as host defense peptides, are widely spread in nature from prokaryotic organisms to vertebrates (Brown and Hancock 2006). These molecules are conserved elements of the natural immunity and have a broad-ranging activity against infectious agents (Jenssen et al. 2006). They can modulate cell functions such as chemoattraction, gene transcription, and cytokine production or release. Also, these peptides may be involved in wound healing and angiogenesis (Lai and Gallo 2009).

HDPs are generally small molecules currently containing approximately 12–50 amino acid residues (molecular weight generally <10 kDa), cationic (net charge of +2 to +7), and frequently quite hydrophobic (Brown and Hancock 2006; Jenssen et al. 2006; Yount et al. 2006). Many of these peptides suffer different posttranscriptional modifications, with disulfide bond formation and C-terminal amidation as the most common ones (Andreu and Rivas 1998). For example, defensins are a widely distributed family of HDPs characterized by specific rearrangements of disulfide bridges (Selsted and Ouellette 2005). The disulfide bridges improve proteolysis resistance and, in some cases, seem to be fundamental for microbial killing (Tanabe et al. 2007; Wanniarachchi et al. 2011), while in others, they do not appear to be a requirement for direct antimicrobial activity (Mandal et al. 2002; Schroeder et al. 2011). On the other hand, some antimicrobial peptides such as clavanins (Lee et al. 1997) present a C-terminal amidation that confers resistance to proteolysis by carboxypeptidases and increases the cationic charge of the molecule (Andreu and Rivas 1998).

Generally, HDPs are genetically encoded molecules included together in multigenic families such as defensins, cathelicidins, cecropins, and dermaseptins (Patrzykat and Douglas 2005). The expression of these molecules could be constitutive or inducible. In prokaryotes, the production of bacteriocins, which are bacterially produced antimicrobial peptides, is generally regulated by a quorum-sensing mechanism of autoinduction when arriving at a certain cell density (Turovskiy et al. 2007). On the other hand, in eukaryotes, the expression of several HDP genes is regulated in different physiological stages: infection (Diamond and Bevins 1994), injury or inflammation (Dorschner et al. 2001), and stress (Aberg et al. 2007). That situation depends on the stimulation and the cell type and it is controlled or synchronized with the expression of other elements of natural immunity and inflammation (Braff and Gallo 2006; Selsted and Ouellette 2005).

According to their secondary structure in solution, these molecules can be generally classified into one of four structural classes: (1) α-helix, (2) β-sheet stabilized by two or three disulfide bridges, (3) extended structures with one or more predominant residues (like tryptophan and proline rich), and (4) loop owing to the presence of a single disulfide bridge (Jenssen et al. 2006; Lai and Gallo 2009). Many of these peptides exist in a relatively unstructured conformation in solution and fold into their ultimate arrangement when interacting with the unique environment of biological membranes (Tossi et al. 2000). The capability to interact with lipid bilayers is essential for the diverse antimicrobial activities of HDPs, although complete membrane destabilization is not always required (Zasloff 2002). The enormous potential of HDPs to interact with biological membranes might bring some concerns on the possible toxicity of HDP-based therapy to host cells, and thus, the selectivity of these molecules constitutes an important issue.

5.3 MECHANISM OF ACTION OF HDPs

Regarding HDPs' mechanism of action, not only are they antibacterial molecules (even against strains resistant to conventional antibiotics) (Miyakawa et al. 1996; Saiman et al. 2001), they could also have antimicrobial activity against fungi (Silva et al. 2014), enveloped viruses like HIV and influenza (Chang et al. 2005; Salvatore et al. 2007), and protozoan parasites as important as *Plasmodium falciparum* (Gelhaus et al. 2008), *Trypanosoma cruzi*, and *Leishmania braziliensis* (Lofgren et al. 2007). Moreover, their toxicity toward cancerous cells has been documented (Do et al. 2014; Hsu et al. 2011). This antimicrobial activity could be by direct action against microbial cells or by an indirect activation of cells from the innate immune system at the site of infection (Alba et al. 2012; López-Abarrategui and Otero-Gonzalez 2013).

HDPs can deal with the lipid bilayer producing cellular death by different mechanisms: (1) changing membrane potential (Westerhoff et al. 1989), (2) transmembrane pore formation (Matsuzaki 1998), (3) modifying the current distribution of membrane lipids with destabilization of membrane structure (Matsuzaki et al. 1996), (4) triggering lethal processes such as the induction of autolytic enzymes (Bierbaum and Sahl 1985), and (5) striking crucial intracellular targets after membrane penetration (Cudic and Otvos 2002; De Brucker et al. 2011; Scocchi et al. 2011). All these different routes underlying the direct antimicrobial activity of HDPs require an initial interaction with biological bilayers, and therefore, the use of model membranes has been widely accepted for learning about this interaction (Hancock and Rozek 2002). The different models proposed can be divided into transmembrane pore models, which imply the formation of actual membrane pores or nonpore ones instead (Wimley and Hristova 2011).

Furthermore, even when the nonspecificity between the interaction of HDPs and membrane lipids has been widely accepted, recent data reveal a more complex scenario. The affinity of some defensins for some specific microbial lipids has been demonstrated (Wilmes et al. 2011). *In vitro*, the inhibition of cell wall synthesis combined with binding experiments and nuclear magnetic resonance spectroscopy demonstrated that Plectasin, a fungal defensin produced by *Pseudoplectania nigrella*, inhibits the growth of Gram-positive bacteria through the binding to

the cell wall precursor Lipid II (Schneider et al. 2010). Also, the inhibition of *Staphylococcus aureus* growing by the interaction of the invertebrate defensins Cg-Defh1, Cg-Defh2, and Cg-Defm, from the oyster *Crassostrea gigas*, with Lipid II has been demonstrated (Schmitt et al. 2010). On the other hand, the binding of the plant defensins DmAMP1 and RsAFP2 to sphingolipids has been demonstrated (Aerts et al. 2007; Thevissen et al. 2003). All these data confirmed the existence of the specific interaction of HDPs with components of microbial membranes. This type of interaction was derived from a novel antimicrobial mechanism that could be conserved between different species.

As has been discussed, many of the mechanisms proposed for describing HDP–membrane interaction are derived from experimental data achieved with model membrane, and therefore, the conclusions might be limited. Also, the relevance of these considerations is limited in relation to *in vivo* infection experiments where the pharmacokinetic properties of peptides (Brinch et al. 2009), serum inhibition of peptide activity (Selsted and Ouellette 2005), and microbial resistance mechanisms (Kraus and Peschel 2006) could influence the active doses needed to eliminate the infection. For example, according to Selsted and Ouellette (2005), under physiological conditions, it is necessary for 1–10 mg/ml of defensin to be fungicidal, conditions only found locally in polymorphonuclear leukocytes, in phagolysosomes, and in the lumen of the crypts of Lieberkuhn. For this reason, it is not practical to consider a peptide-based therapy relying only on its direct antimicrobial activities. Instead, features like immunomodulation could complement and enhance the overall action of such peptides against infections. This balance could be critical in the success of peptide-based therapies.

5.4 HDP THERAPEUTICS

One of the main pitfalls for the utilization of HDPs for bacterial control is peptide inactivation by proteinases. HDP proteolytic inactivation is a real resistance mechanism shared by multiple human pathogens. In an interesting study using group A streptococci also known as GAS, the secretion of at least two factors, cysteine protease SpeB (Schmidtchen et al. 2002) and streptococcal inhibitor of complement (Frick et al. 2003), enabled *in vitro* inactivation of LL-37. Such hypothesis was also proven by Johansson et al. (2008) by using GAS *in vivo* models clearly showing SpeB-mediated LL-37 inactivation representing a bacterial resistance mechanism at severely infected tissue sites. Furthermore, as mentioned earlier, it is notable that HDPs' biological activities are frequently missing at physiologically significant concentrations of glycosaminoglycans, salt, and serum (Afacan et al. 2012). However, despite their enormous potential, are those peptides really effective at *in vivo* models? This is an important question to ask, since the *in vitro* activity does not reveal too much about the real activities of HDPs.

In this context, several *in vivo* trials using animal models have been performed in the last few years (Hilchie et al. 2013). Interestingly, the addition of HDP peptides before initiating infection in a mouse leads to infection reduction (Nijnik et al. 2010; Scott et al. 2007). Moreover, although an extremely weak antimicrobial activity, HNP-1 was able to protect mice from *S. aureus* and *Klebsiella pneumoniae*

infections (Nizet et al. 2001). In principle, anti-infective HDPs' capability could be related to their ability to manipulate immune-cell functions (indirect activation of the innate immune system), direct antimicrobial activities, or a combination of both functions. Nevertheless, once bactericidal properties are mainly lost under physiological conditions (Afacan et al. 2012; Selsted and Ouellette 2005), it has been suggested that such peptides are anti-infective because of their immunomodulatory properties. This proposition was reinforced when the innate defense regulator peptide IDR-1, derived from bovine bactenecin, did not show *in vitro* antibacterial activities. However, such a peptide was extremely protective in several mouse models of bacterial infections (Scott et al. 2007). Surprisingly, such a peptide was protective when delivered topically or systemically through subcutaneous, intraperitoneal, and intravenous means and also effective when utilized after or before bacterial challenge. Undeniably, IDR-1 was capable of stimulating bacterial clearance by acting directly on the host innate immune response, decreasing tumor necrosis factor, and enhancing chemokine production including monocyte chemotactic protein-1.

Additionally, other IDR peptides including IDR-HH2, IDR-1002, and IDR-1018 were also effective in controlling *S. aureus* infections (Achtman et al. 2012; Rivas-Santiago et al. 2013). Moreover, IDR-1002 was also able to protect mice from invasive *Escherichia coli* infection but was unable to control *Mycobacterium tuberculosis* infections. Otherwise, IDR-HH2 and IDR-1018 were capable of reducing bacterial counts in mouse models of drug-sensitive and multidrug-resistant *M. tuberculosis* infections (Rivas-Santiago et al. 2013). Such data clearly suggest that structure and activity are clearly related, although it could be impossible to cross the specificity information for each bacterium. IDR-1018 was also evaluated in diabetic and nondiabetic wound-healing models (Steinstraesser et al. 2012). In such reports, IDR-1018 was compared to LL-37 and HDP-derived wound-healing peptide HB-107. IDR-1018 was suggestively less cytotoxic when compared to LL-37 or HB-107. Surprisingly, although there is complete inefficacy of IDR-1018 to control bacterial colonization, a significant improvement in wound healing in *S. aureus*–infected porcine and nondiabetic models was observed, improving the potential of IDRs and HDPs as pharmaceutical drugs.

Moreover, some studies have focused on polyalanine peptides. Among them is the multifunctional peptide Pa-MAP derived from the polar fish *Pleuronectes americanus* (Migliolo et al. 2012). Pa-MAP was evaluated in intraperitoneally infected mice with a sublethal concentration of *E. coli* (Teixeira et al. 2013), exhibiting the capability to prevent *E. coli* infection and the upsurge in mice survival, being as effective as ampicillin. In addition, mice treated with Pa-MAP have their weight loss reverted. Interestingly, despite other peptides having shown their main *in vivo* bactericidal activities related to immune response, no immunomodulatory activity was observed in such reports, suggesting that bacterial clearance activity obtained could be related to a direct bactericidal effect.

Another option is to discover not only novel peptides but also novel delivery techniques. For example, Ghali et al. (2009) established the viability of protein delivery via microvascular free flap gene therapy, challenging such approach for recalcitrant infections. In this context, authors investigated the LL37 production delivered by *ex vivo* transduction of the rodent superficial inferior epigastric free flap containing

Ad/CMV-LL37. A vascular permeabilizing agent, vascular endothelial growth factor, was also administered during transduction with adenoviral vectors in an effort to enhance transduction efficiency. Moreover, a rodent model of chronic wound infection sewn with bioluminescent *S. aureus* was utilized. Data obtained showed a significant reduction in bacterial loads from infected catheters owing to the expression of LL37 for 14 days, increasing bacterial clearance. Another option consists in the utilization of transgenic mice expressing HDPs. In this field, transgenic mice expressing human beta defensin 2 on different tissues including the intestine, trachea, lung, and skin were more resistant to *S. aureus* infection, despite not reaching complete protection (Zhang et al. 2006).

The pharmacological potentials are enormous and *in vivo* trials are just beginning. At the moment, the *in vivo* peptide activities are completely unpredictable and HDPs that show amazing *in vitro* activities could be extremely pharmacologically useless. Otherwise, peptides that show inconsiderable activities at bioassays could be amazing anti-infectives when challenged at *in vivo* models. The future of HDPs, as pharmaceutical drugs, is a complete open field and totally under construction.

Despite the success of HDPs in preclinical models, the clinical results of these molecules have not been good enough to approve them for medical use. There is no doubt that alternatives to augment the stability, efficacy, and biodistribution of HDPs are needed. Different nanosystems have emerged to develop biomedical applications (Kingsley et al. 2006; Seil and Webster 2012). In fact, some of them have been used to potentiate the activity of HDPs (Brandelli 2012; Urban et al. 2012).

5.5 NANOPARTICLES

Nanoparticles (metals, semiconductors, polymers, and magnets) have a size of between 1 and 100 nm and are characterized by large surface-to-volume ratio, and from this fact, a large fraction of their atoms are located at the surface with unsaturated coordination environments. These atoms are active species able to catalyze redox reactions and to form coordination bonds with surface guest molecules. These are probably the most attractive properties of nanoparticles for their application in biotechnology. For instance, the presence in transition metal oxide nanoparticles of partially naked surface metal sites explains their enzyme-like properties and microbial activity (Biju 2014; Ul-Islam et al. 2014). The coordination chemistry at the nanoparticle surface supports the possibility of their functionalization and conjugation to biomolecules to form programmable molecular engines with unique multifunctional properties, including molecular recognition, target-oriented biosensing, remote-guided nanodevices, and drug and gene transport and delivery, among others. The particles' core properties usually help in their interaction with external agents (e.g., they receive energy from an external source and liberate it in the form of heat into the biological environment). The hyperthermia treatment of cancer tumors using magnetic nanoparticles and a variable external magnetic field and the photodynamic therapy are good examples in that sense (Reddy et al. 2012; Vatansever et al. 2013). All these potential applications of nanoparticles in biomedical biotechnology will be briefly discussed below, with emphasis on the role of nanoparticles as antimicrobial agents conjugated or not with HDPs.

5.6 NANOPARTICLE PREPARATION, FUNCTIONALIZATION, AND BIOCONJUGATION

Nanoparticles, independently of their nature (metals, semiconductors, polymers, and magnets; inorganic, organic, and inorganic–organic hybrid), are obtained by two preparative routes: (1) bottom-up procedures, commonly using colloidal and coordination chemistry, molecular beam epitaxy, laser ablation, chemical vapor deposition, and so on; and (2) top-down routes, for example, milling or grinding (Faramarzi and Sadighi 2012; Sweet et al. 2012). Chemical routes, particularly those involving colloidal chemistry, allow appropriate control of particle size and shape, nanocrystal morphology, and surface active sites, which are structural features required for their biological applications, both *in vitro* and *in vivo*, including functionalization and bioconjugation. In this context, functionalization comprises the incorporation, through acid–base reactions or coordination interactions, of molecular species to the particles' surface to allow their conjugation to biomolecules or to provide functional properties for molecular recognition and separation, target modification and activation/inactivation, drug and gene delivery, bioimaging and sensing, and so forth (Schrofel et al. 2014). The chemical routes are also appropriate for tailoring the particles' composition, size, and shape, which determine their optical, electrical, thermal, and magnetic properties (Akbarzadeh et al. 2012).

The functionalization and bioconjugation of nanoparticles depend on their nature and surface composition. In this sense, silica nanoparticles illustrate the importance of the surface chemistry having an appropriate interface for the functionalization and bioconjugation processes. Silica is a hydrophilic and biocompatible material; it is transparent to the optical region of the electromagnetic spectral region and has a high physical and chemical stability. In addition, the preparative methods to obtain silica nanoparticles and mesoporous silica nanostructures from sol-gel and colloidal routes are well established, including the control of the pore size and shape (Trewyn et al. 2007). A wide diversity of reactive functional species (including carbonyl, primary and secondary amine, hydroxyl, azido, and alkyl halogen groups) can be incorporated into the silica surface during the preparative process through postsynthesis surface modification (Figure 5.1). Such reactive surface species, with both basic and acidic features, serve as anchoring sites for organic and biological molecules, including fluorescent markers, antibodies, nucleic acids, peptides, and proteins. Biological molecules have heteroatomic sites with basic and acidic characteristics able to form chemical bonds with the silica surface reactive groups. Practically all the available bioconjugation protocols are applicable for silica nanostructures (Trewyn et al. 2007).

The abovementioned features of silica support its incorporation to core@shell nanostructures, particularly when the core has limited chemical stability in biological environments or liberates toxic species (e.g., CdS luminescent quantum dots) or when the availability of an easily functionalizable shell is recommended. Quantum dots are semiconductor nanostructures (ZnS, ZnSe, ZnTe; CdS, CdSe, CdTe, ZnO, etc.) that absorb light in the UV-vis spectral region, promoting an electron from the valence band to the conduction band. Part of the energy of the resulting excited state is then transferred to the network solid as heat through the excitation of phonons

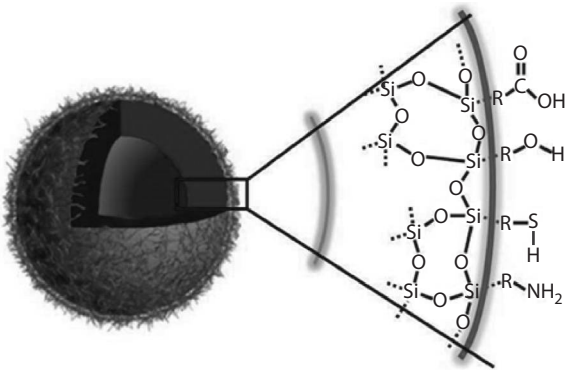

FIGURE 5.1 A nanoparticle (metallic, semiconducting, magnetic) with a surface layer (shell) of silica to facilitate its functionalization and bioconjugation. Indicated are four possible functional groups (R-NH_2, R-SH, R-OH, and R-CO_2H).

and that remaining is emitted as fluorescence, from trap states, in the visible and NIR spectral region. Both the spectral region where the quantum dot absorbs light and the region where it emits can be tuned, controlling its size and shape. The quantum efficiency for the emission process and the line width of the emitted light are controllable parameters through particle surface passivation incorporating a shell of appropriate molecular species (e.g., trioctylphosphine, trioctylphosphine oxide, etc.). The most important property of quantum dots, in the context of biomedical applications, is the tunable photoluminescence. This determines their application as fluorescent labels in analytical biochemistry, imaging, and sensing (Chinnathambi et al. 2014). Compared with organic fluorescent markers, quantum dots show a higher luminescence, with narrow emission lines, and are practically free of photo bleaching. The synthesis of high-quality quantum dots commonly involves the use of organic reagents, and the resulting nanoparticles are obtained with a surface layer of organic molecules with hydrophobic character. In consequence, their functionalization and bioconjugation must be preceded by an exchange of surface ligands. In order to prepare the surface for its further functionalization and bioconjugation, the ligand exchange reaction must produce a hydrophilic surface with available reactive species, for example, carboxyl, amine, and hydroxyl groups (Jiang et al. 2014; Kuzyniak et al. 2014). The degradation of quantum dots and the heavy metal liberation into the biological environment can be prevented, creating a thin surface coating of silica (Figure 5.1) and using the above-discussed benefits of this last material for the nanoparticles' functionalization and conjugation or growing a layer at the surface of a less toxic semiconductor, for instance, ZnX (X = O, S, Se, Te). In this last case, at the surface, an alloy or solid solution of the two semiconductors is formed, which also helps tune the emission spectra (Estévez-Hernández et al. 2012). Quantum dots have found applications in photodynamic therapy as well through their conjugation to photosensitizers. The energy of the excited state is transferred to the anchored sensitizer molecule and finally used for the singlet oxygen and reactive oxygen species (ROS) production (Biju et al. 2010).

Metallic nanoparticles have attracted considerable attention for biological applications not only because of their metal bioactivity but also because of their optical properties based on light absorption through surface plasmon resonance (Schrofel et al. 2014). Nanoparticles of silver and gold show a strong plasmon resonance effect in the UV-vis-NIR spectral region, which is tunable by modifying particle size and morphology and through the chemical species anchored to their surface. Plasmon resonance light absorption leads to a color change for the incident radiation, which is utilized for imaging the region where the nanoparticles are located. If the nanoparticles are functionalized with target-oriented groups, for example a monoclonal antibody, the optical spectra can be used for sensing purposes. The energy absorbed by the particle from the incident radiation is then partially liberated to the particle environment in the form of heat. This is exploited in photothermal cancer therapy. A fraction of the adsorbed light is reemitted as bright vis-NIR photoluminescence, which provides an image of the sites where the nanoparticles have been adsorbed (Schrofel et al. 2014). The surface plasmon resonance also supports surface-enhanced Raman spectroscopy, a technique with an ultrahigh sensitivity for adsorbed species on metallic surface. The adsorption of a molecule on a metal surface leads to an increase of approximately 10^{12} times in the Raman signal intensity. This technique allows the sensing of a single molecule when it is adsorbed on a metal nanoparticle, for instance, silver or gold. Such ultrahigh sensitivity makes possible the detection of quite a small amount of molecular pathological markers in biological fluids or tissues and for *in vitro* and *in vivo* marker-specific imaging (Schlücker 2010). Metals show strong x-ray absorption, and from this fact, the target-oriented biosensing and linking of metal nanoparticles to specific biological sites in tissues serve as contrast agent in x-ray imaging. Similar to quantum dots, as-synthesized metal nanoparticles usually require capping ligand exchange to allow their functionalization and bioconjugation. From such ligand exchange, nanoparticles with a wide diversity of reactive surface groups can be prepared, including carboxyl acids, amines, azides, maleimides, alcohols, amino acids, peptides, and proteins. These molecules have heteroatomic sites able to form a chemical bond with the surface metal sites. For instance, the conjugation of gold nanoparticles exchanged with thiolated carboxylic acids to peptides, proteins, amino acids, and antibodies is relatively simple (Shah et al. 2014).

Related to the large availability of carbon in nature, the wide diversity of carbon nanostructures (nanotubes, fullerenes, graphenes, nanodiamonds, and onions), and unique physical properties (strong absorption of light in the UV-vis-NIR spectral region, NIR photoluminescence, high photothermal response, tunable electrical behavior, etc.), the potential applications of carbon-based materials in biology and pharmacy are receiving increasing attention (Moon et al. 2009). Similar to silica, the surface of carbon nanostructures is functionalizable with a wide variety of reactive groups, among them, carboxylic acids, amides, phenols, alkyl halides, and alcohols. The conjugation to biomolecules and the incorporation in the surface of target-oriented molecules, for example antibodies, allow the conjugate sorption on specific molecular or tissue region for imaging, sensing, drug and gene delivery, or target modification. Target modification involves the therapy for cancer and microbial infections, through photothermal and oxidative stress effects. Carbon nanostructures show exceptional abilities for production of ROS, including singlet oxygen. The photothermal ablation

of targeted cancer cells and tumors using NIR light or radiofrequency is an attractive research area currently in progress for the application of functionalized and bioconjugated carbon nanostructures (Moon et al. 2009).

Magnetic nanoparticles have additional attractive characteristics, compared with their metallic, semiconducting, silica- or carbon-based analogs, for biological and pharmaceutical applications. Magnetic nanoparticles are susceptible to remote guiding through an external magnetic field, and once they are located on the selected target, the application of an oscillating magnetic field permits local heating for target modification, including cellular death. This last possibility supports their potential application for cancer treatment. When magnetic nanoparticles are subjected to a time-varying magnetic field of low frequency (250 Hz), the particles receive energy from the applied magnetic field through orientation of their magnetic dipole moments, which is then liberated into the biological environment when they are disoriented (Figure 5.2). By this mechanism, the tumor is warmed to a temperature of 42°C–45°C, where the tumor cells are irreversibly damaged (Reddy et al. 2012). A heating time of approximately 30 min could be sufficient to destroy a tumor. Hyperthermia treatment of cancer is being intensively studied. It supposes that the magnetic nanoparticles are directed to and adsorbed by the target sites, by using an external magnetic field as well as through their functionalization with highly specific target-recognizing molecules, for example, monoclonal antibodies.

Nanoparticles of iron oxides, particularly of magnetite (Fe_3O_4), are the commonly used magnetic nanoparticles for biological and pharmaceutical applications. The preparation of this iron oxide is relatively simple; it can be obtained practically free of toxic by-products, and its tolerance for biological systems is high. The partially naked iron sites and the OH groups found at magnetite particles' surface are appropriate reactive species for their functionalization or bioconjugation. When a higher versatility for the functionalization is required or a higher chemical stability is recommended, a core–shell system with silica, $Fe_3O_4@SiO_2$, could be prepared (Figure 5.1). The applications of magnetic nanoparticles in biomedical sciences are diverse (Erathodiyil and Ying 2011; Lopez-Abarrategui et al. 2013; Reddy et al. 2012): analytical biochemistry for separation and concentration of analytes and

FIGURE 5.2 Localized drug delivery using magnetic nanoparticles and a magnetic tape.

molecular markers from biological fluids, drug and biomolecule transport and delivery in *in vivo* systems, chemoembolization for tumor vessel blockage in order to induce hypoxia, magnetically guided radioimmunotherapy, and as contrast agent for magnetic resonance imaging through the tuning of the transverse relaxation time.

5.7 ANTIMICROBIAL PROPERTIES OF NANOPARTICLES

The antimicrobial activity of different types of nanoparticles has been demonstrated. In fact, some metals like zinc, silver, and copper exhibit antibacterial properties in their bulk form. The antimicrobial effect of these metals increases at nanoscale dimensions (Seil and Webster 2012). The mechanism of action of these nanoparticles varies from one nanoparticle to another. Although these antimicrobial mechanisms are not fully understood, some are related to the damage caused by the physical structure of the nanoparticles, whereas others are associated with the release of metal ions from nanoparticle surfaces. For instance, the mode of action of silver nanoparticles is reliant on Ag+ ions, which interact with respiratory enzymes, the electron transport system, and DNA to inhibit microbial growth (Li et al. 2006).

The antibacterial activity against *S. aureus* of polysaccharide-reduced silver nanoparticles has been confirmed (Kemp et al. 2009). Furthermore, the inhibition (*in vitro* and *in vivo*) of different viruses by silver nanoparticles has also been established (Baram-Pinto et al. 2009; Rai et al. 2014; Xiang et al. 2013). Recently, an antimicrobial peptide (G3R6TAT) was conjugated to silver nanoparticles (Liu et al. 2013). The conjugated peptide showed enhanced antibacterial and antifungal activity compared with silver nanoparticles. These results suggest that silver nanoparticles have potential in antimicrobial therapeutic applications.

Antibacterial as well as antifungal activity has also been documented for ZnO nanoparticles (Jones et al. 2008; Siddique et al. 2013; Wahab et al. 2010). The mechanism of action of many of these ZnO nanoparticles involves the production of ROS (Dutta et al. 2012; Raghupathi et al. 2011).

Iron oxide is not antibacterial in its bulk form but may exhibit antibacterial properties as nanoparticles. For example, magnetite nanoparticles coated with quaternary ammonium were bactericidal against *E. coli* (Dong et al. 2011). Furthermore, bacitracin-conjugated iron oxide (Fe_3O_4) nanoparticles have shown higher antimicrobial activity against both Gram-positive and Gram-negative organisms, in comparison with the bacitracin peptide (Zhang et al. 2012). Because of this improved activity, conjugated magnetic nanoparticles allow lower dosages and collateral effects of the antibiotic. Moreover, cell cytotoxicity tests indicate that bacitracin magnetic nanoparticles show very low cytotoxicity to human fibroblast cells, even at relatively high concentrations. Because of their antibacterial effect and magnetism, the conjugated bacitracin magnetic nanoparticles have potential application in magnetic-targeting biomedical applications. On the other hand, the synthesis of multimodal nanoparticles with magnetic core and silver shell showed very significant antibacterial and antifungal activities against 10 tested bacterial strains (minimal inhibitory concentration [MIC] from 15.6 to 125 mg/l) and 4 *Candida* species (MIC from 1.9 to 31.3 mg/l) (Prucek et al. 2011). In another experiment, the HDP LL37 was conjugated to magnetic nickel nanoparticles coated with a nanolayer biofilm of polyacrylic acid. An

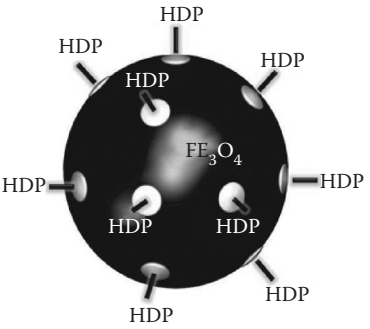

FIGURE 5.3 A possible conjugation route for HDPs to nanoparticles. The presence of gold islands or shell at the nanoparticles allows the peptide molecules to link to the nanoparticles' surface. This leads to the formation of multifunctional nanostructures.

effective bactericidal activity was demonstrated for the conjugated peptide (Chen et al. 2009). Similarly, citric acid–functionalized manganese ferrites were conjugated with the antifungal peptide Cmp5. The antimicrobial activity of the conjugated ferrites was higher than their bulk counterparts (Lopez-Abarrategui et al. 2013).

These results reinforce the importance of nanochemistry to fight different infectious diseases. On the other hand, the high affinity of gold for amino acids containing molecules can be used for HDP conjugation to magnetic and semiconductor nanoparticles, through the postsynthesis incorporation of a thin shell or islands of gold on the surface of magnetite or CdS, for instance (Odio et al. 2014). The adsorption of gold atoms on thiol-capped magnetite nanoparticles produces gold islands at their surface. The conjugation of peptides to such gold-containing nanostructures leads to the formation of multifunctional conjugates (Figure 5.3).

5.8 CONCLUSIONS

Despite the broad spectrum activity of HDPs and success in preclinical models, the clinical results of these molecules have not been good enough to approve them for medical use. Alternatives to reduce the toxicity and increase the stability, efficacy, and biodistribution of HDPs are urgently needed. Nanotechnology could provide a solution for these problems. Nanoparticles could be a perfect carrier for HDPs because of their multifunctional activities. Because the antimicrobial properties of both molecules have different mechanisms of action, the conjugated peptides (HDP–NP) could have an enhanced activity. The preparation and study of these multifunctional conjugates open unpredictable opportunities and result in biological and pharmacological applications to combating infectious diseases.

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