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Review Article

Nanoparticle applications in intracellular infections

Judith Torres[1*], Tatiana Lara[2], Marcelo Grijalva[2, 3]

[1] *Facultad de Ciencias Médicas, Universidad Central del Ecuador, PO BOX 170136, Quito, Ecuador.*

[2] *Centro de Nanociencia y Nanotecnología, Universidad de las Fuerzas Armadas ESPE, PO BOX 175231B, Sangolquí, Ecuador.*

[3] *Departamento de Ciencias de la Vida y la Agricultura, Universidad de las Fuerzas Armadas ESPE, PO BOX 175231B, Sangolquí, Ecuador.*

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*Address for Correspondence:

Judith Torres, Facultad de Ciencias Médicas, Universidad Central del Ecuador, PO BOX 170136, Quito, Ecuador.

Abstract

Objective: To review selected recent studies on the therapeutic applications of nanoparticles and nanoparticle-conjugated antimicrobials as new therapeutic alternatives for a variety of intracellular infections.

Data Sources: Recent published papers on nanoparticles and intracellular infections, including bacterial, viral, and parasitic diseases in humans. The PubMed database was used as the main source. Publicly available papers were retrieved.

Summary of Contents: Therapeutic failure in intracellular infections is a challenging clinical problem. Antibiotics, anti-parasitics, antivirals, and other drugs might not reach effective levels in intracellular compartments; these agents in high doses might become toxic and may show undesirable effects. Researchers have been looking for alternative strategies for antimicrobials to reach intracellular spaces. The development of novel drugs and release mechanisms is currently a research priority in infectious diseases. With this background, new approaches such as those based on nanotechnology, including the fabrication of drug nanocarriers, are of increasing interest to researchers and clinicians. The goal for nanocarriers is to provide controlled release of drugs into cellular compartments with high selectivity, higher efficiency, better therapeutic outcomes, less toxicity, and more rational dosing schemes compared to traditional ones. Biocompatibility of nanocarriers may ensure affinity to the reticuloendothelial and immunological systems, which might facilitate drug delivery into intracellular compartments.

Conclusion: Nanoparticle systems have great potential in infectious diseases, particularly in difficult-to-treat infections, such as those caused by intracellular pathogens. These systems have been tested with several drugs, enzymes, genes, and peptides, showing long half-lives due to their hydrophilic coatings. The optimization of nanoparticle-based drug delivery systems has improved our understanding of the different mechanisms underlying biological interactions and the engineering of even more complex nanoparticles.

Keywords: Nanoparticles, intracellular infections, novel therapeutics.

Introduction

Nanoscience and nanotechnology involve the synthesis, characterization, and applications of materials and structures at the nanoscale, that is, in the size range from 1 to 100 nm. Due to their optical, electronic, magnetic, and chemical properties and also to their potential technological applications, a large number and a wide variety of nanoparticle types have been developed and studied in recent years [1,2]

Many scientific studies based on nanotechnology have provided the foundation for the development of a range of applications in medicine and biology. This technology can help with the development of nanoscale systems for use in health-related conditions [3]. Nanoparticles have an important role in molecular diagnosis, the treatment and monitoring of various diseases, cancer therapy, thrombosis, molecular imaging, *in vivo* drug release, and antimicrobial activity [4]. Nanoparticles are particularly advantageous due to their size, surface area, and capabilities of *in vivo* release of medications, along with particular optical, magnetic, and electrical properties [5,6]. Another advantage is the ability of nanoparticles to internalize into cells, which improves the efficacy of therapies in many pathological conditions [7].

Among a growing number of NPs being synthesized and studied, metallic nanoparticles have been long considered of interest due to their rapid synthesis and wide range of potential applications. Several researchers have synthesized zinc, titanium, copper, gold, platinum, and silver nanoparticles using different strategies and synthesis techniques.

Biological synthesis of nanoparticles is an alternative strategy to the commonly used physical and chemical methods. Biological synthesis uses several organisms such as microorganisms or plants, as reducers and stabilizing agents for NPs production [8]. In the same way, eco-friendly methods use organisms and their naturally produced compounds such as plant extracts, biopolymers, etc. It has been observed that the structures obtained by these methods can be stable and maintain specific size, shape, and chemical composition,

offering high monodispersibility and an efficient scale of production [1,2 , 8]. In biological or green synthesis, biological compounds can be used alone or combined with chemical and physical strategies, with the following advantages: 1) The use of non-toxic chemicals for the reduction and stabilization of NPs. 2) Optimized energy usage, because these processes usually do not require high pressure and energy conditions, and, 3) Ease of scale up production [9].

The green nanoparticle synthesis method uses plant extracts as an alternative to chemical and physical methods, which might be toxic, flammable, hazardous, and not friendly to the environment. Studies show that nanoparticles of gold and silver metals have the size, morphology, stability, and physical-chemical properties that might be tuned by experimental conditions and allow for physical, chemical, optical, and electronic properties control of nanoscopic materials [2]. Furthermore, these noble metals have a strong surface plasmon that exerts oscillating resonances and might constitute good candidates for biological synthesis [2]. In a recent study, the extract of *Trianthema decandra* (native plant of India) in aqueous solution was used to reduce gold chloride and silver nitrate to their metallic form of gold and silver. The characterization was performed by ultraviolet-visible (UV – VIS) spectroscopy, electron microscopy, X-ray diffraction (XRD), and FTIR (Fourier Transform Infrared Spectroscopy). The authors found that the silver and gold nanoparticles had strong antibacterial activity on *Yersinia enterocolitica*, *Proteus vulgaris*, *Escherichia coli*, *Staphylococcus aureus*, and *Streptococcus faecalis*. Growth inhibition of *Candida albicans* was twice as high with silver nanoparticles as with gold [2].

Silver and gold nanoparticles might be used in the medical industry, in applications such as topical to prevent infections in wounds and burns. Gold nanoparticles are toxic to bacteria and fungi. The contact between the surface of the microorganism and the nanoparticles causes damage in the cell, producing destruction of the flagella and, stimulating the production of biofilm and aggregation within it. Another study compared the size of gold and silver nanoparticles and concluded that the intense antimicrobial activity of silver nanoparticles is related to their smaller size in relation to gold nanoparticles [2].

Research by Mukunthan KS, et al focused on the green synthesis of silver nanoparticles (Ag-NPs) using an extract from *Catharanthus roseus*. The obtained nanoparticles were characterized by UV - VIS spectroscopy, electron microscopy scanning (SEM), X - ray energy dispersivity (EDX), and X - ray diffraction measurement. The formation and stability of the Ag-NPs in colloidal aqueous solution were confirmed by UV-VIS. The solution exhibited a yellow-brown color due to the reduction of the silver ion, which indicates the formation of AgNPs. SEM images in this study showed spherical nanoparticles with a diameter between 48 and 67 nm. The EDX analysis confirmed the presence of elementary silver. X-ray diffraction studies confirmed the crystalline nature of the particles and the XRD pattern showed the Braggs reflection number related to the central surface of the silver structure. The antimicrobial activity of the Ag-NPs was assessed in clinical isolates of *Staphylocccus aureus*, *Eschericha coli, Klebsiella pneumoniae, Bacillus cereus*, and *Pseudomonas aeruginosa*. Inhibition diameters were compared against a control of chloramphenicol (10 ng / mL), with a maximum inhibition diameter of 11 mm in *Bacillus cereus* and a minimum zone of 6 mm in the other bacteria. The antimicrobial effect was explained by the penetration of nanoparticles into the bacterial wall and by the modulation of signaling through dephosphorylation of peptide substrates in tyrosine residues [6].

An important component of the defense against an infectious process is the mononuclear phagocyte system of the reticuloendothelial system, which can be evaded by bacteria [10]. Some studies suggest that macrophages could act as transporters of pathogenic microorganisms such as *M. tuberculosis*, *Burkholderia pseudomallei*, *S. aureus*, and *L. monocytogenes* as they prevent the formation of the phagolysosome [10].

Some bacteria and fungi can synthesize silver nanoparticles. Bacterial systems are an excellent option for the biosynthesis of nanoparticles since genetic manipulation is relatively easy [11]. The use of nanoparticles as a strategy to prevent the production of biofilms in implants or medical procedures is a new potential therapeutic strategy [12].

Nanotechnology systems have shown to be potentially useful as efficient carriers for a diversity of molecules to target sites in specific organs and tissues. With this, the therapeutic effect of anticancer, antiviral, antibiotic, and antifungal molecules has been increased [3]. To understand the antibiotic nanostructure, however, it is necessary to know the interactions among their components, study the process of drug encapsulation and its subsequent mechanism of action, the functional activities, and the pharmacokinetic and pharmacodynamic properties [3]. In fact, antimicrobial peptides and their different structures have allowed the development of different carriers such as liposomes, dendritic polymers, and solid –cover nanoparticles or carbon nanotubes [3].

The characterization of nanoparticles is carried out using a variety of techniques that include electron microscopy, atomic force microscopy (AFM), dynamic light scattering (DLS), X – ray diffraction (XRD), Fournier-transform infra-red (F-TIR), scanning electron microscopy - SEM, transmission electron microscopy -TEM and combinations of these techniques, among many others. The above techniques allow the study of the size, shape, composition, surface charge, and other physical and chemical characteristics of the nanostructures [3,10,13]. It is well known that data onNPs characteristics play an important role in predicting the behavior and/or interaction of NPs with microorganisms, intracellular components and body compartments and might even help in predicting the NPs´potential toxicity [14]. For example, the possible mechanisms of action of NPs and their association with microbial membranes [3,13,15]. Thus, it is important to discuss how different NPs enter cells, evade the immune system, and act against intracellular infections at a specific site.

Immune responses to nanostructures

Nanosystems intended for drug delivery should be evaluated based on their therapeutic efficacy and biosecurity. For this purpose, not only the antimicrobial activity needs to be taken into account, but also the possible modulation of the immune response (immune stimulators or suppressors), [10,11] and the potential cellular toxicity (immunotoxics) [3,13,15]. The activation of an immune response (innate or adaptive) relies on nanoparticle interactions with body fluids, peptides,proteins and their recognition by phagocytes, (in the innate, non-specific immune response), and/ or antibodies (in the adaptive, specific immune system) [17,18]. The interaction between proteins/peptides, body fluids, and NPs , which mostly depends on energy, polarity, charge, composition, and morphology of the nanostructures [15], can lead to changes in the integrity and morphology of the NPs and their subsequent recognition by the immune system.

In this aspect, nanoparticles conjugated with peptides or polymers, have physical and chemical properties that are particularly well suited for drug delivery systems [3,10,13]. For instance, polymer-drug conjugation provides protection against the host's immune system responses and different

polymers can be used as coatings for nanoantibiotics, including vinyl polymers, polysaccharides, poly amino acids, and polyethylene glycol (Figure 1). Natural polymers also, such as agarose, chitosan, chitin derivatives, and alginates, have been used in the development of nanodrugs, due to their high stability and degradability, providing an adequate level of biocompatibility to the nanostructure [3,10].

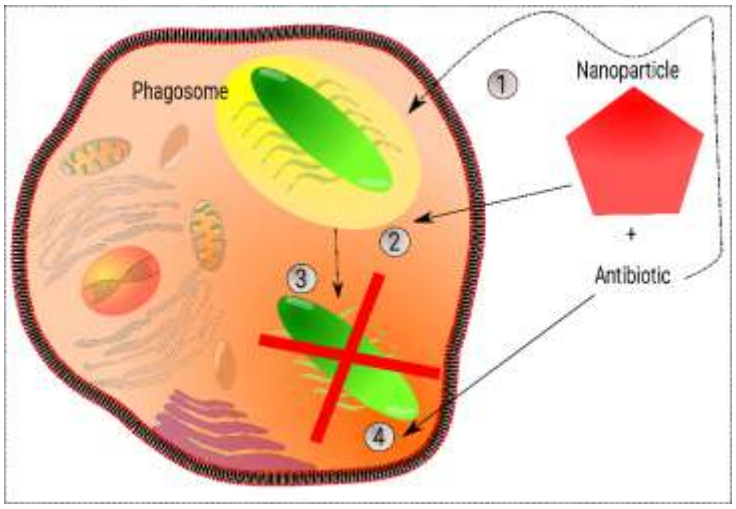

Figure 1: Nanostructures linked to antibiotics (nanoantibiotics). [1] Nanoparticle uptake and internalization via different mechanisms of pinocytosis. [2] Phagosomal uptake, [3] Degradation of nanoparticles and, [4] Controlled release of encapsulated antibiotics.

The immune response can be modulated both by the release rate of the drug and by the immunomodulatory effects associated with some nanoparticles [3,13,15]. To understand the immunomodulatory effect, the mechanism of internalization of nanoparticles must be considered. Due to the size of nanoparticles (usually between 10-500nm) and their charge and/or polar composition, they cannot passively cross the cell membrane. NPs uptake mostly occurs through pinocytosis (active endocytosis) with four possible mechanisms (Figure 2); (i) endocytosis mediated by clathrin, (ii) endocytosis mediated by caveolae, (iii) clathrin- and caveolae-independent endocytosis, (iv) and macropinocytosis [14].

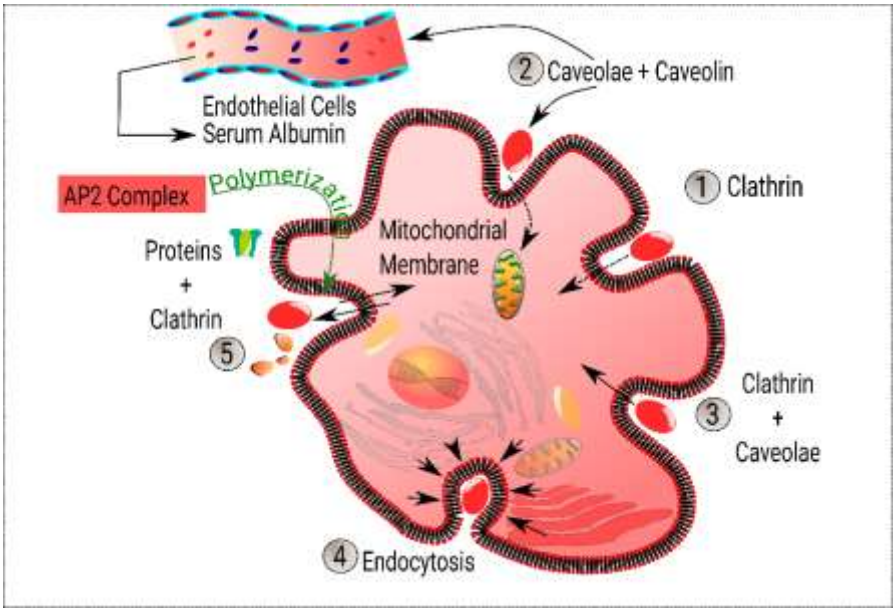

Figure 2: Mechanisms of nanoparticle uptake by cells. See explanation in the text.

Clathrin and caveolae – mediated endocytosis work through a receptor-ligand pathway, where the ligand, along with NPs, binds to the receptor and changes its conformation, resulting in endocytic vesicles translocated into the cell [19]. (i) The internalization pathway mediated by clathrin (CME) has been studied at the molecular level. CME involves numerous proteins such as clathrin adapter (AP2) and accessory proteins (AP180, epsin and SNX9) that initiate the polymerization of clathrin in the plasma membrane, which results in the formation of a vesicle that contributes to the internalization or externalization of materials. [20] In general terms, 40 to 200 nm nanoparticles and most likely the ones with polymeric coats (PEG. PLGA, SiO_2, etc.) could enter cells via CME [14,20]. (ii) The pathway mediated by caveolae is characterized by the presence of caveolin and different accessory proteins. NPs of 20-100nm NPs and of different types, have shown to internalize using this mechanism [20]. Also, caveolae-dependent endocytosis is associated with

mitochondrial membrane activity and the transit of serum albumin, particularly in endothelial cells [7]. (iii) In cells without clatrhin or caveolae proteins, clathrin and caveolae-independent endocytosis (CCIE) is carried out. This mechanism has five subcategories that mostly require a lipid composition and helps in the transport of growth hormones, GPI, IL-2 among others including folate NPs.[21]. (iv) The macropinocytosis process creates the largest vesicles among pinocytosis subtypes (0.2-5µm) [21]. Macropinocytosis does not take into account the size or shape of the NPs and takes place in almost all non-phagocytic cell types [20]. Therefore, this process has a nonspecific uptake where a fluid or micrometric particle gets in contact with a cell and causes its deformation and encapsulation (Figure 3) [14].

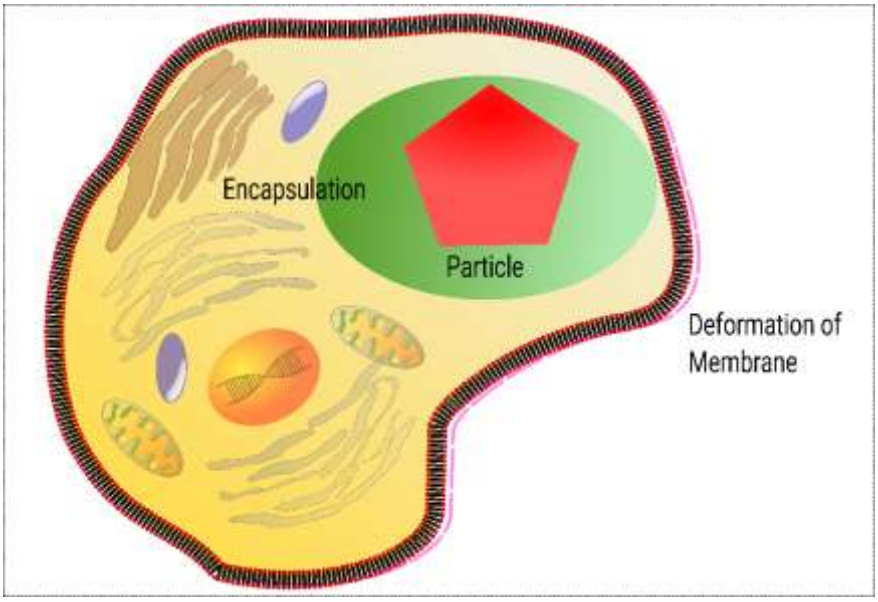

Figure 3: Nanoparticle cell uptake by macropinocytosis with subsequent membrane deformation. For details see text above.

Besides eliciting immune responses, NPs of different sizes and compositions may mediate regulated cell death pathways and cytotoxicity. Though cytotoxicity may be deleterious for the cell, targeted toxicity could be helpful in specific applications. We will review below the main mechanisms associated with cell death induced by diverse NPs.

Autophagy is a cellular pathway that facilitates some immune responses. This mechanism prevents the invasion of pathogens by transporting transferrin between the cytosol and the lysosomal vesicles [7]. The interaction between autophagy and signaling of the innate immune system is modulated by a cytokine-mediated inflammatory response to microbial stimulation. Autophagy enhances the presentation of antigens to T lymphocytes through major histocompatibility complex type I molecules [16]. Due to these beneficial antimicrobial effects, the induction of autophagy could be one of the targets for new drugs and vaccines. NPs can be used as inducers of autophagy, the most common mechanism being the interaction of the NPs with p62 protein during ubiquitination or by adding intracellular proteins that facilitate pathogen invasion, which causes activation of the LC3 pathway (Figure 4) [16,22]. The bioconjugation of nanoparticles with proteins is considered an efficient method for transporting molecules to cells and also for controlling the number of protein molecules transported. Some NPs, for example, those based on polyamidoamine (PAMA- M), gold, iron, and Buckminster fullerene (C60) have shown to induce autophagy [16,22,23].

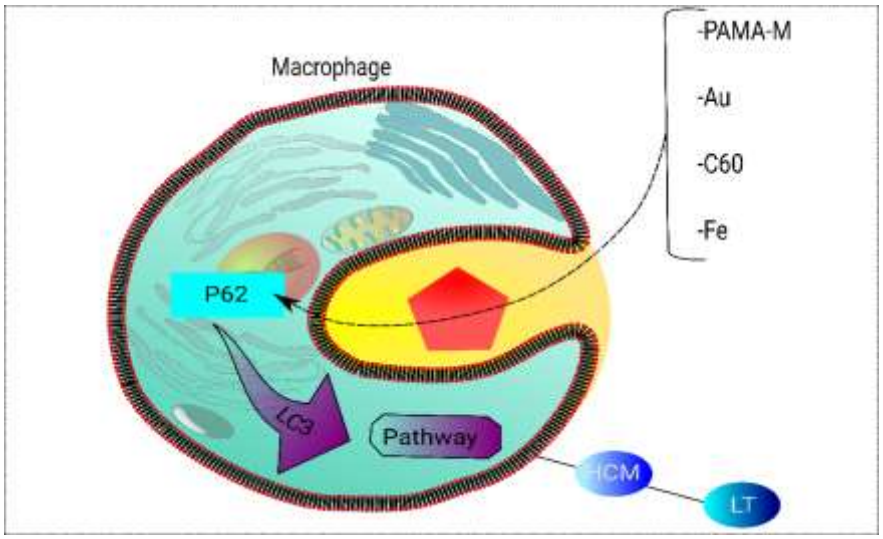

Figure 4: Nanoparticle-induced autophagy in macrophages and activation of the LC3 pathway. For details see text above.

Nanoparticles and intracellular infections

Nanotechnology becomes an alternative strategy in addressing the re-emergence of infectious diseases, especially those caused by antibiotic-resistant Gram-negative bacteria. There are many problems associated with bacterial resistance due to the presence of intracellular pathogens, hence the interest in antibiotics that could reach intracellular compartments and clear them of pathogens. For example, beta-lactam antibiotics are widely used for the treatment of infections caused by different pathogens. However, they have low penetration in phagocytic cells. Also, they have a non-desirable acidic pH, because antibiotics need neutral or physiological pH to act. Therefore, transporters of beta-lactam antibiotics such as NPs that cross cell membranes at physiological pH have been studied [24].

Now a days NPs are being widely studied because of their potential to act as drug carriers or antimicrobials on their own (e.g. silver NPs). Moreover, NPs can reach the desired location, cross the cell membrane by diffusion or endocytosis, and had a convenient controlled/sustained release of the drug [25]. Additionally, the size of the nanoparticles favors appropriate interaction between microbial membranes and inorganic agents, such as metals or metaloxides, A good example of this is zinc oxide nanoparticles (ZnO – NPs) which can interact with lipid membranes and disorganize the structure of the membrane and bacterial wall, destabilizing and causing death. ZnO at the nanoscale enters the cell and causes the production of free radicals which damage DNA, cell membranes, and proteins and inhibits bacterial growth. [26]. Table 1 summarizes selected studies of nanostructures developed to treat intracellular infections.

Table 1: Summary of selected research on nanostructures developed to treat intracellular infections.

APPLICATION	TYPE OF NANOSTRUCTURE	TARGET SITE	MAIN RESULTS	REFERENCE
Autophagy	Polyamindoanime (PAMA- M), gold, iron and buckministerfullernce (C60)	Immune system	Antimicrobial activity	(Beyzay F. Z. H., 2017), (Stern ST., 2008), (English L., 2009).
Prevent production of biofilms in implants or medical procedures.	Nanoparticles	Biofilms	Biofilm inhibition	(Markowsk K., 2013)
Gram-negative bacteria	Zinc oxide nanoparticles	Lipid membrane.	Antimicrobial activity	(Salem W. L. D., 2015)
Gram-positive and Gram-negative bacteria	Benzylpenicillin (PNG) with squalene (Sq) nanoparticles	Phagocytic cells with bacteria	Antimicrobial activity	(Semiramoth N., 2012)
S. aureus	BOPIDY- labeled nanoparticles	Endosomal and lysosomal vesicles	Lower infection rate	(Semiramoth N., 2012)
Gram-positive and Gram-negative bacteria, fungi	Green nanoparticles from *Trianthema decandra* with silver or gold	Antimicrobial assays	Antimicrobial activity	(Geenthalakkshmi R S. D., 2012)
Gram-positive and Gram-negative bacteria	Green nanoparticle from *Catharanthus roseus* with silver	Bacterial wall and modulation of signaling by dephosphorylation of peptide substrates in tyrosine residues	Antimicrobial activity	Mukunthan KS., 2011)
Endophthalmitis	Tripolyphosphate (TTP)-daptomycin nanoparticles, chitosan nanoparticles and a mixture of TTP and chitosan-coated daptomycin nanoparticles.	Bacterial inhibition	Antimicrobial activity	(Silva N C., 2013)
Post-operative orthopedic infections *Staphylococcus aureus* (MRSA).	Silver nanoparticles covered with tiopronin	Bacterial inhibition	Antimicrobial activity	Procovich P., 2013)
Chronic infections with *Salmonella* spp.	Chitosan-ceftriaxone nanoparticles	Antibacterial effect	Antimicrobial activity	(Zaki N.M., 2012)
***Lysteria monocytogenes, Salmonella enteritidis,* and *Eschericha coli* O 157: H7.**	ZnO-PVP nanoparticles	Antibacterial effect	Antimicrobial activity	(Jin T., 2009)
E. coli* O157: H7 and *Salmonella enteritidis	ZnO QDs, ZnO PVP	Bacterial growth	Antimicrobial activity	(Jin T., 2009
Salmonella* and *Lysteria	Nanoparticles with gentamycin	Formation of vacuoles of *Salmonella* and	Antimicrobial	(Ranjan A. P. N.,

monocytogenes		*Listeria* in the cell cytoplasm.	activity	2009)
L. monocytogenes	Amoxicillin nanoparticles covered with microspheres of PLGA.	Decrease infection rate	Antimicrobial activity	(Farazuddin M. A. M., 2010)
L. monocyotgenes	Amoxicilin nanoparticles coated with albumin microspheres (HSA), amoxicillin nanoparticles covered with PLGA microspheres.	Decreased infection rate	Antimicrobial activity	(Farazuddin M. A. M., 2010)
Brucella sp	Gentamicin AOT PLGA nanoparticles	Lysosomal compartment reservoir systems	Antimicrobial activity	(Imbuluzqueta E. G. C., 2013) (Seleem M. M. P., 2009)
Brucella sp	Nanoparticles from Gantrez ® and Nanosil radiolabeled with 99 m TC and coated with *Brucella ovis* antigens.	Immune response	Vaccines	(Sanchez Martinez M., 2013)
Mycobacterium tuberculosis	ROS / RNS - generating nanoparticles	Cytokine production by macrophages, lysosome-phagosome fusion.	Release of the drug towards lysosomes	(Dube A., 2014)
Chlamydia trachomatis D - K	G4-PANAM dendrimers conjugated with azithromycin.	Intracellular growth phase	Antimicrobial effect	(Mishra M.K., 2011)
Chlamydia trachomatis	Encapsulated liposomes with DOX or CZX	Elementary intracellular bodies	Antimicrobial effect	(Ikeda-Dantsujiy., 2011)
***C. trachomatis* serotypes A, B, Ba and C**	Liposomes with BGs	Specific immune response in ocular surface.	Vaccines	(Inic-Kanada A., 2015)
C. pneumoniae	(PLGA) HFA227 propellant	Acute or chronic infections, elementary intracellular bodies.	Antimicrobial effect	(Bharatwaj B., 2010)
Leishmania	Curcumin nanoparticles coated with mannosylated chitosan (Cur - MCNPs)	Phagolysosomal vacuole with parasitic intracellular amastigotes.	Antiparasitic effect	(Chaubey P., 2014)
Leishmania amazonensis* and *Leishmania chagasi	Chitosan (NQ), chitosan - chondroitin sulfate (NQC) and nanoparticles of Chitosan - chondroitin sulfate and amphotericin B (NQC - AmpB).	Reduction of intracellular parasite load	Antiparasitic effect	(Riberio T G., 2014)
Leishmania infantum	β-aescin in PLGA nanoparticles.	Macrophages infected with amastigotes, phagolysosome.	Antiparasitic effect	Van de Ven H. V. M., 2012)
Leishmania infantum	Bisnaphthalimidopropyl- polymer nanoparticles.	Amastigotes of *L. infantum.*	Antiparasitic effect	Beyzay F. Z. H., 2017)
***Leishmania major*.**	Nanoparticles with cysteine peptidase A (CPA) and cysteine peptidase B (CPB).	Immune response and vaccine preparation.	Prevention	Beyzay F. Z. H., 2017)
L. major	α-albumin with CPA and CPB nanoparticles.	Internalization of the nanoparticles into macrophages.	Vaccines	Beyzay F. Z. H., 2017)
HIV-1	Dapavirine-PLC nanoparticles.	Antiviral action in certain cell types.	Antiviral action	(Neves J., 2012)
Plasmodium falciparum	Cryptolepine-gelatin hydrochloride nanoparticles.	Reduced hemolytic effect in the host.	Antiparasitic effect	(Kuntoworbe N., 2012)

NPs applications in intracellular infections

Salmonella spp, is a facultative intracellular bacterium that uses many mechanisms of evasion before defense cells and establishes specialized intracellular niches that might be sequestered from the immune system, often producing chronic infections. Salmonellosis is difficult to treat because antibiotics may have poor diffusion into intracellular retention compartments [27,28]. The use of chitosan in the composition of NPs associated with ceftriaxone is a potential system for antibiotic release to cells infected with Salmonella. In a study by Zaki, it was shown that chitosan-ceftriaxone nanoparticles are internalized via Caco-2 to J774.2 macrophages with rapid kinetics, indicating synergy between chitosan and the antibiotic,increasing their antibacterial effect against *Salmonella typhimurium* [29].

In an investigation by Jin et al. (2009), quantum dots of zinc oxide (ZnO QDs) were synthesized from oxide Zn (ZnO), polystyrene / ZnO (PS / ZnO) in films, and from polyvinylpyrrolidone (PVP)-covered ZnO. The antimicrobial activity of these different nanoparticles was evaluated in cultures of *Listeria monocytogenes, Salmonella enteritidis*, and *Eschericha coli* O 157: H7. For *L. monocytogenes*, ZnO-PVP NPs showed to be more effective in causing bacterial inhibition, whereas treatment with ZnO PS on film showed no effect on bacterial growth. Similar results were seen for *E. coli* O157: H7 and *Salmonella enteritidis*. The authors applied the QDs onto liquid egg white contaminated with bacteria, demonstrating a decrease in Lysteria colonies in egg white. Therefore, ZnO QDs were more effective than nanoparticles of ZnO PVP. Additionally, the authors cultured *S. enteritidis* in egg white with different concentrations of ZnO QDs and without them (control), demonstrating a significant and dose-dependent reduction of bacterial growth in presence of QDs nanoparticles. They concluded that nanotechnology provides an opportunity to solve many problems such as achieving an effective concentration of ZnO. Nanoparticles have a small size and large surface area, and the ability to penetrate through cell membranes and ensure rapid antibacterial activity, and lastly might be uniformly distributed within a medium with low sedimentation and applying minimal dosages [30].

L. monocytogenes, an intracellular pathogen, is associated with food-borne diseases, newborn infections, septicemia, and meningitis in patients with weakened immune systems. The antibiotics used for these pathologies are aminopenicillins, which may not reach intracellular compartments, causing therapeutic failure. Ranjan et al. (2009) conducted a study on the stability and functionality of nanostructures in physiological medium for the release of gentamicin into macrophages. The strategy was to incorporate hydrophobic molecules of high- molecular weight polyphenylene oxide (PPO) in nanoparticle coatings to facilitate transport within macrophages and determine their activity in the vacuoles of *Salmonella* and *Listeria* in the cytoplasm. A quantitative reduction of *Listeria* suggested that its intracellular localization was influenced by the action of the NPs. The encapsulation of gentamicin in nanostructures was related to the presence of amphiphilic or amphipathic surfaces that modulate the path into macrophages. This phenomenon can improve the therapeutic efficacy at the cellular level [31]. Other authors used amoxicillin alone and amoxicillin-coated poly (lactic-co-glycolic acid) (PLGA) microspheres to study the therapeutic efficacy in Swiss albino mice infected with *L. monocytogenes.* The treatment of amoxicillin alone failed, while the treatment with amoxicillin NPs-PLGA significantly decreased the infection in the study animals [32]. In another study, investigators tested amoxicillin NPs coated with human serum albumin (HSA) or with PLGA in mice infected with *L. monocytogenes.* It was shown that amoxicillin NPs coated with PLGA were more effective than albumin-coated amoxicillin NPs [33].

Brucella sp, is a facultative intracellular pathogen that invades mainly cells and organs of the mononuclear phagocytic system, such as macrophages in the liver and spleen, causing chronic infections [28,34]. The management of human brucellosis is still under study through the *in vitro* evaluation of antibiotics able to reach different compartments. Aminoglycosides have low intracellular activity against *Brucella* sp., butdespite their high hydrophilicity, they poorly reach the lysosomal compartment and have a reduced activity because of the acidic pH [35,36]. Preparations of gentamicin with modified hydrophobicity such as gentamicin 2-ethylhexy sodium sulfosuccinate (AOT) have been studied in the form of PLGA nanoparticles with encapsulated antibiotic. *In vivo* studies in mice with brucellosis demonstrated that gentamicin AOT in PLGA NPs formulation maintained efficient antimicrobial activity for up to four days in liver and spleen [34]. This preparation forms the so-called nanoplexes, which can act as reservoir systems which in this study showed slow and continuous release of the antibiotic into target organs after a two-dose treatment [37]. Other authors used the water / oil evaporation method for synthesis of PLGA NPs containing gentamicin, with a size smaller than 350 nm. The efficiency of the encapsulation of gentamicin depends on the type of polymer used in its preparation and on the *in vitro* release of the antibiotic in continuous patterns of PLGA 502 H. The PLGA 502H NPs were phagocytosed by J774 murine monocytes after intravenous administration in mice and release towards the liver and spleen where Brucella is usually found was observed [38].

The optimization of NPs synthesized from Gantrez ® and Nanosil and radiolabeled with 99 m TC, coated with *Brucella ovis* (Man - NP - HS) allowed the study of biodistribution in mice after ocular administration. The localization of these NPs in the nasal mucosa and gastrointestinal tract induced a rapid protective immune response. These findings have led to consider NPs as potential platforms for the development of vaccines for brucellosis [39].

Mycobacteria and Chlamydia

Mycobacterium tuberculosis is an intracellular resident pathogen that possesses evasion mechanisms of the host immune system and that can also induce different degrees of suppression of the host defense system. In a 2014 study, Dubee et al., evaluated the use of ROS / RNS- generating nanoparticles and stimulators of cytokine production by macrophages. CS - PLGA and 1,3 β glucan were tested on the surface of NPs to promote fusion of the lysosome with the phagosome and increase the concentration of Ca^{2+}, in turn promoting the release of the drug towards lysosomes and attacking *Mycobacterium tuberculosis* [40].

Chlamydiae are obligate intracellular bacteria, which lodge inside epithelial cells, required for their survival and growth. The species of Chlamydia that causes diseases in humans are *C. trachomatis* and *C. pneumoniae*. *C. trachomatis* is the main cause of sexually transmitted diseases including non-gonococcal urethritis and epididymitis in men, cervicitis and pelvic inflammatory disease in women, and conjunctivitis and pneumonia in newborns of women with active genital infection. *C. pneumoniae* is a primary respiratory pathogen and frequently causes community-acquired pneumonia in adults and children. Chlamydia infections are treated with antibiotics that interfere with DNA and protein synthesis, such as tetracyclines, macrolides, and quinolones. The treatment is not always effective due to the development of resistance and to the intracellular lodging characteristics of this pathogen.

Hence, the importance of seeking new strategies to combat these infections [41].

Chlamydia trachomatis, serovar D - K, which is sexually transmitted, has a biphasic cycle with an infectious elementary body (CE) and a reticular body (CR). Exposure to certain adverse conditions can induce a state of persistence or a stress response. In the persistence phase, Chlamydiae are viable but not infectious, which could lead to therapeutic failure. *Chlamydia trachomatis´*extracellular infectious elementary bodies (EB) (0. 2 µm) enter the host genital epithelial cells within an endosome. Following the fusion of elementary bodies, endosomes develop into larger (0.8µm), replicative, non -infectious bodies (reticular bodies, RBs). RBs use ATP and metabolites from the host cell to grow and subsequently divide within a cytoplasmic inclusion. RBs mature into infectious elementary bodies and are released from the infected host cell [42]. Exposure to certain adverse conditions can divert the developmental cycle into a state referred to as persistence or Chlamydial stress response. In persistence or stress, Chlamydia remain viable, but non –infectious [43].

In a study by Kinter, β lactams amoxicillin, clavulanic acid, ampicillin, and carbenicillin were tested in different concentrations against Chlamydia and abnormal inclusions with persistence / stress response were observed. It was demonstrated that Chlamydia remains viable based on the continuity of detection of its genome and the accumulation of pre rRNA 16S. To this, the ability to regain infectivity after drug withdrawal, is added. If stress factors are removed, Chlamydia returns to its normal cycle. [44].

Azithromycin is effective against Gram-positive and Gram-negative bacteria. Conjugation with fourth-generation polyamidoamine dendrimers (G4-PANAM) has been tested to increase the efficacy of the free drug as a microbicide or to attenuate the growth of *C. trachomatis* in the intracellular growth phase [45]. G4-PANAM dendrimers conjugated with azithromycin were used, with an esterase as a binding molecule. Azithromycin was modified in the 2' position to obtain reactivity and functionality for their release from the dendrimer. It was shown that dendrimers alone enter the cells and do not produce enough stress to induce persistence of the infection. Conjugates of dendrimers with azithromycin have a higher antimicrobial effect compared to the antibiotic alone, the conjugate is more effective in decreasing the number and size of inclusions. Conjugates of azithromycin with G4-PANAM were deemed promissory as a new treatment for genital and non-genital Chlamydia infections [45].

In another study the authors determined the minimum inhibitory concentrations (MIC) of liposomes with encapsulated doxycycline (DOX) and ceftriaxone (CZX). Cell cultures were treated with antibiotics (encapsulated and non-encapsulated) and then infected with Chlamydia and subjected to sonication. After 48 to 72 hours of infection, the inclusion bodies were observed by fluorescence microscopy. The authors of this study demonstrated that the application of sonicated encapsulated liposomes did not cause significant effects on cell viability and Chlamydial infection. Doxycycline at MIC of 0.015 µg/ml or ceftriaxone 1.0 µg/ml in combination with the encapsulated liposomes reduced the number of inclusions compared to the antibiotic alone. This study suggests the possibility of using liposome-encapsulated antibiotics as release model to eradicate intracellular infections [46].

Infections with *C. trachomatis* serotypes A, B, Ba, and C can lead to trachoma, the most important cause of blindness in developing countries. The disease initiates with an acute inflammatory response mediated by Th1 cells and IgA antibodies. The use of "bacterial ghost" BGs as carrier "vaccines" that produce immune responses associated with conjunctival lymphoid tissue (CALT) was explored in a study by Inic-Kanada. The authors used BGs, which are liposomal particles that contain on their surface several structures that intervene in the recognition of antigens and that enable the nanoparticle to function as an antigen-presenting agent. BGs consist of two membranes separated by a periplasmic space and different oligosaccharide membranes anchored to this structure. In recombinant BGs, proteins and nucleic acid sequences are in the periplasmic space. The authors tested the liposomes for the release of nucleic acids into the ocular mucosae, getting a good tolerance of human conjunctival epithelial cells (CECs) *in vitro* and in guinea pigs´ conjunctiva *in vivo*. This tolerance is important to ensure that an innate response is elicited and that specific chlamydial antigenic subunits are expressed. [47].

The treatment of pulmonary infections caused by *Chlamydia pneumoniae*, may in the future include inhalation formulations with nanocarriers capable of reaching the lungs with facilitated access to intracellular bacterial inclusions. In a study by Bharatwaj, PLGA nanoparticles exhibited good biodegradability, good dispersion in water and the ability to transport themselves in the cellular cytosol. PLGA NPs were marked with fluorescence (6-coumarin), to track and localize the nanoparticles by fluorescence microscopy in Calu-3 cells infected with *C. pneumoniae*. Subsequently, the aerosol in which the nanoparticles were placed (with HFA227 propellant), was prepared and characterized to test stability and dispersion. A poor stability in the disperser was found, for which the nanoparticles were modified with doses of pressurized inhalers (pMDis). This aerosol was tested using the Anderson Cascade Impactor (ACI) scale [48] which measures the fraction of fine particles that reach the respiratory tract. It was demonstrated that the respiratory route, through inhalation preparations associated with nanoparticles, is an excellent alternative in the treatment of acute or chronic infections caused by *C. pneumoniae* [48].

Bacterial endophthalmitis is an ocular inflammation of the posterior eye compartment caused by pathogenic microorganisms. The infection cause vision alterations and irreversible damage of retinal photoreceptor cells and subsequent blindness. The bacteria most involved are *Staphylococcus aureus*, coagulase-negative Staphylococci, *Streptococcus viridans*,and other cocci and Gram-negative bacilli such as *Escherichia coli.* Treatment of these infections is difficult due to the innate protective barriers such as cornea and conjunctiva, the latter with an epithelium that has unions that limit the entry of substances into the eye, among them molecules such as antibiotics. In one study naked daptomycin NPs, tripolyphosphate (TPP) -chitosan coated daptomycin NPs and daptomycin in solution were prepared and tested for stability in the presence of lysozyme and mucin. Minimum inhibitory concentrations (MIC) of the different preparations were estimated against different strains of *Staphylococcus aureus*, with coated nanoparticles exerting greater bacterial inhibition. The nanoparticles showed appropriate characteristics for ocular topical administration and the antimicrobial activity of daptomycin was preserved after encapsulation. The authors of the study concluded that the ionic interaction between mucin and chitosan nanoparticles is favorable for prolongation of the contact time between the nanoparticles and the mucus film on the ocular surface, thus contributing to a better absorption of the drug. Lysozyme had no effect on the integrity of the chitosan nanoparticles. The presence, however, of mucin significantly altered the loading surface of the nanoparticles, changing its charge from positive to negative, change that might impair the interaction and association of the nanoparticles in the ocular mucosae [49].

Sémiramoth N., et al synthesized new amphiphilic derivatives of benzylpenicillin (PNG) with squalene (Sq) at both sensitive and non-sensitive pH, with two bioconjuncts of different nature between the hydrophobic chain of penicillin G. In the first component, the binding to each group proved to be less labile than in the latter, more hydrolysable, depending on pH, and demonstrating stability of the nanoparticles. Subsequently, the nanostructures were tested on the J774 cell line of murine macrophages which were infected with *S. aureus*. A significant destruction of *S. aureus* was determined through the LysoTracker lysosomal marker. This marker acts on endosomal and lysosomal vesicles, initiating endocytosis and cell internalization pathways. Subsequently, a propidium iodide (red) marker was used to stain infected macrophages and the nanoparticles were marked with BOPIDY (green) and observed through confocal microscopy. The study showcases an interesting perspective to treat bacterial infections [24].

In orthopedic surgery, in spite of pre and post-operative antibiotics being usually administered and with bone cement additionally embeded in antibiotics, postoperative infections are still common. An interesting study used Ag-NPs covered with tiopronin and tested their antimicrobial effectiveness against strains of methicillin-resistant *Staphylococcus aureus* (MRSA). Both, NPs with encapsulated bone cement and free nanoparticles were tested in the above study. The Ag-NPs with tiopronin bound to bone cement molecules demonstrated excellent stability due to the binding of the hydrogen chains and to the negative charge repulsion between the protonated groups of the terminal carboxyl groups. The assembly stability of these solid NPs stored for long periods was demonstrated. The nanoparticles were not affected in their size, which ensures an easy handling while compatibility with biological systems allows the incorporation of bone cement [50].

Leishmanial infections

Leishmania, an intracellular parasite, eludes the action of anti-parasitic drugs by penetrating into cells, leading to antimicrobial resistance and infection reactivation. For a complete eradication of intracellular parasites, the concentration of the drug in macrophages must be greater than the minimum inhibitory concentration. Subsequently, the internalization of the drug transporter complex would cause greater accumulation of the drug in the target cells and potential damage to healthy cells, which are sensitive to the toxic effects of the drugs. Chitosan is a natural polymer obtained by alkaline de-acetylation of chitin. Chitosan is non-toxic, biocompatible, and biodegradable and can be metabolized by human enzymes such as lysozyme. The conjugation of mannose as ligand with chitosan, could be used for the design of selective transport systems for drug release in macrophages and the spleen [51]. Curcumin (CUR) is a natural anti-inflammatory, anti-proliferative, anti-mutagenic, anti-cancer and cytotoxic compound for some strains of *Leishmania major*, *L. tropica*, and *Linfantum*. In a recent study, curcumin nanoparticles coated with mannosylated chitosan (Cur - MCNPs) were prepared. These NPs were tested on the surface of the reticuloendothelial system. The in vitro findings revealed that Cur - MCPNs have anti - parasitic activity against intracellular amastigotes [51].

Riveiro et al. used another approach against leshmaniasis. asystem for the release of amphotericin B-NPs (AmpB-NPs) using a polyelectrolyte technique in which two oppositely charged polymers with antihistamines chitosan (Cs) and chondroitin sulftate (ChS) was developedThe NPs were loaded positively with chitosan and negatively with chondroitin sulfate. Three different nanoparticles were prepared: chitosan (NQ), chitosan - chondroitin sulfate (NQC) , and nanoparticles of chitosan - chondroitin sulfate and amphotericin B (NQC - AmpB). The Nps haddiameters of 79, 104 and 136 nm respectively and a polydispersity index of 0.2. The Z potential of the nanoparticles indicated a positive charge on the surface. Transmission electron microscopy revealed spherical nanoparticles with a smooth surface. Cs, ChS and NQ, NQC and NQC - Amp B promised to be effective against promastigotes of *Leishmania amazonensis* and *Leishmania chagasi*, particularly NQC - Amp B nanoparticles. Interestingly, null or low hemolytic activity was also observed in serotype O + when authors probed only AmpB and AmpB-NPs, and they concluded that AmpB is more toxic. In this study, the authors additionally tested NPs on cells infected with *L. amanzonensis*, obtaining reductions of the intracellular parasites of 24%, 31%, 55% 66%, 90% and 89% for Ch, ChS, NQ, NQC and NQC - AmpB and pure Amp B, respectively. The data presented indicate that NQC-Amp B NPs might be useful as alternative therapies in the treatment of leishmaniasis due to their low toxicity in mammalian cells [52].

β-aescin, a saponin purified from *Aesculus hippocatanum*, has strong intracellular activity against amastigotes of *Leshmania infantum*, but shows high levels of cellular toxicity. In order to decrease this saponin toxicity, polymeric PLGA-NPs have been developed for the release of β-aescin. In one study, the preparation of B-aescin in PLGA nanoparticles showed a concentration of 1 to 18 times inside the macrophages compared to β-aescin alone, indicating that the drug transporter is effective and that it is possible to reduce the cytotoxicity of β-aescin while maintaining the anti-leishmanial activity of the drug [53]. In a following work, the authors modified the synthesis of PLGA saponin β-aescin NPs combining the emulsification / solvent evaporation technique with saponification by alternating phases. Immunofluorescence and confocal microscopy studies showed that nano-microparticles (with diameters of 200-400 nm) presented to *L. infantum* amastigote-infected J774A.1 macrophages were mobilized through lysosomes and decreased the number of amastigotes. In addition, PLGA nanoparticles remained active within lysosomes and diffusion of the drug from the phagolysosome was significant [54]. PLGA and other colloidal transporters could be the basis of potential new therapeutic schemes for visceral leishmaniasis. The formulation of nanoparticles with β – aescin holds promise as anti-leshmanial treatment due to its *in vitro* physico-chemical characteristics.

The use of bisnaphthalimidopropyl (BNIP) derivates has been described as *Leshmania silent* and *Leshmania infantum* inhibitors [55]. However, BNIP has low solubility in water and is toxic in high doses. Costa et al. encapsulated BNIP in a biodegradable polymer (PLGA), to increase the solubility of BNIP and reduce its cytotoxicity. The authors tested the NPs on cellular models of human leukemia monocytes (THP1), the murine macrophage J744 cell line, and mouse fibroblasts line L929. Then NPs were also tested *in vivo* in mice infected with previously cultured promastigotes of *L. infantum* MHOM / MA / 67 / ITMAP-263. Five groups were used in this study: (I) negative control, (II) NPsg / PLGA, (III) 1 mg/Kg of amphotericin as positive control, (IV) 1 mg/Kg BNIPD/AOCT, and, (V) 1 mg/Kg of PLGA NPs loaded with BNIPD. After 3 days of treatment, mice were euthanized, and liver and spleen samples drawn for quantification of parasites. BNIPD/AOCT-loaded PLGA NPs showed complete internalization in host cells, especially in macrophages and fibroblasts. Interestingly, drug-loaded NPs proved to be more effective and selective in destroying amastigotes of *L. infantum* in the macrophage model than in the fibroblast model. These results were compared to those from the *in vivo* model (BALB/c mice) in phagocytic cells, where large number of NPs were found in the spleen and the liver, demonstrating higher efficiency of the NPs loaded with the drug in reducing the number of intracellular parasites in acute infection [56].

HIV

Dapivirine,a reverse transcriptase inhibitor used topically as antiretroviral has shown high activity against HIV-1 with less toxicity than other anti-retrovirals. Dapivirine has been associated with polycaprolactone (PLC) nanoparticles to induce greater antiviral action in certain cell types [57]. A study by Armiji et al showed that 200 nm nanoparticles of dapivirine-PLC are able to penetrate the cervico-vaginal epithelium [57]. To characterize the release of dapivirin by nanoparticles, the authors used a cell line of human vaginal epithelial cells (VK2 / E6E7), human cervical cells (HeLa), J774A.1monocytes mouse macrophages (Mo / Mac), TZN-bl, PBMCs, and dendritic cells. A microbicidal effect was seen in all cell lines. The intracellular release of dapivirine was evaluated through fluorescence microscopy. In VK2 / E6E7 and HeLa cells the PCL-dapivirine NPs showed a good interaction with cell membranes and were internalized via clathrin-mediated endocytosis. In the TZM-bl cells, less diffusion of the nanoparticles and the drug was observed. In the case of Mo / Mac phagocytic cells, PBMs and dendritic cells, the results were markedly different. An increase in intracellular drug levels was observed when the nanoparticles reached the intracellular space. This leads to the role of passive systems that have special importance in HIV transmission and infection. The incorporation of dapivirine in nanoparticles influences the drug levels observed in each specific cell line [57]. The results of antiretroviral tests indicate high potency of nanoparticle-encapsulated dapivirin with low lethal index 50 (EC50) and sustained antiretroviral activity [57].

Malaria

Malaria is a tropical disease caused by *Plasmodium falciparum*, it has high mortality and low response to different antimalarial drugs. Hence, the development of new drugs and new formulations for the treatment of this disease is necessary [58]. In a study, the authors manufactured gelatin NPs loaded with cryptolepine hydrochloride. These nanoparticles showed low hemolytic effects on erythrocytes. Four formulations with drug and nanoparticles were tested, with cryptolepine alone showing more undesirable effects, in comparison with the NPs [58]. Therefore the nanoparticles of cryptolepine-gelatin hydrochloride reduced the hemolytic effect compared to the drug alone [58].

Concluding remarks

Nanotechnology and medicine

Nanotechnology applications in biology and medicine are currently critical to improve diagnosis and management of several human diseases, including infectious ones. Nanoscale systems could be applied in therapeutic products, provide molecular diagnostics, usually associated with anticancer biomolecules, antivirals, antibiotics and antifungals. In addition, they have shown to be useful as transporters of bioactive molecules for specific targets in affected organs and tissues, thus increasing their therapeutic potential [3].

Nanobiotechnology platforms have showed feasibility for the development of new drugs. The use of nanostructures provides a molecular barrier that shields the bioactive component within the host organism. Biocompatibility with host and pathogen membranes plays an important role in improving therapeutic efficacy. In infectious diseases, one can envision the future of new antibiotics with increased stability, higher efficacy and better release control against different types of pathogens [3].

Nanotransporters in nanoscale structures provide a specific bioactive way of releasing drug molecules to an organ and target tissue, providing higher specificity, better absorption and improved therapeutic potential with reduction of toxicity. In infectious diseases, antibiotics could be administered in lower doses, decreasing side effects and keeping the therapeutic effect for longer times, thus avoiding bacterial resistance [3].

Nano-scale drug preparations may show, however, toxic effects. There is, to date, no consensus on the type and characteristics of assays for assessment of cytotoxicity of nanoscale platforms. The Unites States National Nanotechnology Laboratory at the National Cancer Institute has armonized, along with the European Nanotechnology Laboratory panels for cytotoxicity evaluation of nanostructures intended for medical applications. Among them, the evaluation of DNA damage, and carcinogenesis is of particular importance. The micronuclei test has been useful for the determination of the potential cytotoxicity and genotoxic effects of nanostructures in bone marrow. The effects of cytotoxicity are determined by the frequency of polychromatic erythrocyte while the genotoxic effects are assessed by counting the number of polychromatic micro-nuclei and the generation of normochromatic erythrocytes [3].

In contrast, different studies have shown that nanoformulations might be less toxic than conventional formulations. Nanostructures might not cause instability in chromosomes or cells of the bone marrow, preventing potential genotoxicity as with antifungals [3]. To reduce the cytotoxic effect, it has been observed that the size of the nanostructures must be smaller with a large surface that provides a greater volume of drug uptake [3]. Depending on the preparation method, nanoparticles, nanospheres or nanocapsules can be obtained. Nanocapsules are systems in which drugs are confined to a cavity surrounded by a single polymer membrane while nanospheres are matrix systems in which the drug is physically evenly dispersed. In recent years, biodegradable polymeric nanoparticles, particularly those coated with hydrophilic polymers such as polyethylene glycol (PEG), known as broad circular particles, have been used in the release of drugs because of its ability to circulate for prolonged periods and reach target organs. Applications of PLG-based nanostructures such as DNA carriers in gene therapies and studies on the release of specific proteins, peptides and genes have been developed and published [59].

Nanoparticle-based release systems

A demonstrated useful application of nanoparticles is their role as release systems for molecules and compounds of interest. This due to nanoparticles´ particular size, surface properties, and pharmacological action in specific sites , along with improved therapeutic and dose regimens compared to traditional delivery systems. Liposomes have been used as potential transporters that provide a means of controlled protection to drug degradation, with better efficacy in reaching the target site while reducing toxicity. Liposomal formulations may be limited due to encapsulation efficiency, rapid leakage in aqueous solutions and blood, and poor storage stability. Polymeric nanoparticles seem to offer specific advantages over liposomes. They can increase drug-protein stability and provide better control of the nanoparticle properties. Some of the advantages of using nanoparticles as drug delivery systems include,

1. The characteristics of size and surface can be manipulated and corrected both passively and actively after parenteral administration

2. The control of the release of substances and drugs during transport to the site of action alters the distribution in the

organ and its subsequent degradation, improving therapeutic efficacy and reducing side effects.

3. The controlled release and the particular degradation characteristics are modulated by the constituents of the nanoparticle matrix. Nanoformulation coatings allow the drug to be incorporated into the particle structure without chemical degradation, being this an important factor for preservation of drug activity.

4. Specific ligands may reach specific target sites on the surfaces of nanoparticles.

5. Nanoformulations may be engineered for oral, nasal, parenteral, or intraocular administration [59].

Applications in infectious diseases

Nanoparticle systems have great potential in infectious diseases, particularly in difficult-to-treat infections, such as those caused by intracellular pathogens. These systems have been tested with several drugs, enzymes, genes and peptides, showing long half-lives due to their hydrophilic coatings. The optimization of nanoparticle-based drug delivery systems has improved our understanding of the different mechanisms underlying biological interactions and the engineering of even more complex nanoparticles [59].

The applications of nanoparticle-based drug release systems in the medical area are endless and in several medical areas such as oncology, infectology, traumatology, immunology, vaccine formulation, among others. Most nanoparticles have been tested in cell and animal models, very few have been tested in humans so far. It is expected that in the next few years experimental nanoformulations might be already in clinical trials. The promise of effective new nanotechnology-based therapeutics in intracellular infections is already near the corner for clinicians.

Conflict of interest

The authors declare no conflict of interest.

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