The growing role of nanotechnology in combating infectious disease

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Key words: nanotechnology, antimicrobial, antibiotic, vaccine

Antimicrobial Nanotechnology-Based Drug Delivery Systems

Chitosan. Chitosan is a natural polysaccharide biopolymer derived from chitin, which is the principal structural component of the crustacean exoskeleton. The antimicrobial properties of chitosan result from its polycationic character, which favors interaction with negatively-charged microbial cell walls and cytoplasmic membranes, resulting in decreased osmotic stability, membrane disruption and eventual leakage of intracellular elements.2-4 In addition, chitosan is able to enter the nuclei of bacteria and fungi and inhibit mRNA and protein synthesis by binding to microbial DNA.5 When nano-scaled, chitosan has a higher surface-to-volume ratio, translating into higher surface charge density, increased affinity to bacteria and fungi and greater antimicrobial activity.5 Several studies have demonstrated the efficacy of chitosan nanoparticles against a variety of pathogens, including Gram-negative E. coli and Gram-positive S. aureus.5-6 Chitosan nanoparticles were found to be more effective against these bacteria than chitosan alone,7 acetic acid8 and other antibiotics such as doxycycline.9 In addition, chitosan’s polycationic nature and high affinity to metal allow it to be used as a carrier system and platform stabilizer for a variety of other nanoparticles including silver- and copper-containing nanoparticles,6 nitric oxide-releasing nanoparticles,7 and drug-containing nanoparticles that allows for targeted delivery of various medications.5 Chitosan platforms also augment the antimicrobial properties of these nanoparticles. For example, the antimicrobial efficacy of silver-loaded membranes is enhanced with increasing chitosan contents of up to 70%, resulting in larger zones of inhibition against both S. aureus and E. coli.5 In addition, the mean inhibitory concentrations of AgNPs against S. aureus was significantly decreased upon addition of chitosan to the nanoparticle matrix (AgNPs-9 ± 4 vs. Chitosan-AgNPs-1.25 ± 0.75).8

Metallic nanoparticles. Silver. For centuries, silver (Ag) has been used for the treatment of burns and wounds to prevent infection.9 Although the mechanism of its antimicrobial effects is not entirely known, it has been proposed that silver and silver ions (such as AgNO3) penetrate bacterial cell walls and membranes via interaction with sulfur-containing proteins or thiol groups.10 Once inside the cell, AgNO3 targets and damages bacterial DNA and respiratory enzymes, leading to loss of the cell’s replicating abilities and ultimately cell death.11 The small size and large surface area of silver nanoparticles (AgNPs) makes them better able...
to penetrate bacterial cell walls and membranes, and as a result, the antimicrobial effect is directly dependent on nanoparticle size and shape. Smaller nanoparticles (<10 nm) as well as triangular or truncated nanoparticles are more effective than larger particles that are round or rod-shaped. AgNPs have been shown to be effective against a variety of pathogens, including viruses, fungi (C. albicans), and many bacterial species including E. coli, S. aureus, B. subtilis, and S. typhi. The stronger interaction of AgNPs with microbial surfaces might also allow for the use of lower drug concentrations as compared to current silver agents, and may limit silver’s toxicity. However, these benefits are largely theoretical, and the adverse effects of silver nanoparticles have been less well characterized.

AgNPs have also been found to augment the efficacy of other antimicrobial agents. In particular, the antibiotic activities of penicillin G, amoxicillin, erythromycin, clindamycin and vancomycin increased against S. aureus and E. coli when mixed with AgNPs, with erythromycin having the greatest synergy with the silver. Microbes are also less likely to develop resistance against silver and AgNPs, as their broad range of targets would require multiple and simultaneous compensatory mutations. As a result, silver can be used to arrest bacterial resistance to antibiotics and enhance their efficacy. Given these properties, there are many diverse applications for AgNPs, including coatings on medical devices to prevent microbial colonization, wound dressings and augmentation of antibiotics.

Copper. There is little reported on the mechanism of copper’s antimicrobial activity, but its antifungal and antibacterial properties have been utilized for decades, as evidenced by its use as an antifouling coating in the 19th century. Copper oxide (CuO) is cheaper and more easily mixed with polymers as compared to Ag. As such, the use of CuO nanoparticles (CuO-NPs) as a novel antimicrobial agent has recently been investigated. When compared to AgNPs, CuO-NPs were shown to be less effective against E. coli and methicillin-resistant S. aureus but more effective against B. subtilis, which may be due to copper’s greater interaction with amine and carboxyl groups on the cell surface of this pathogen.

Even though Ag may be a stronger antibacterial agent, Cu potentially has a broader range of activity, especially against fungi. The antimicrobial capacity of copper nanoparticle-loaded polymer thin films (CuNP), which uniformly release copper, has been demonstrated against S. cerevisiae yeast, molds and bacteria including E. coli, S. aureus and Listeria monocytogenes. For all microbes tested, copper nanocomposites resulted in lower counts of colony forming units (CFUs), and this effect was particularly clear with S. cerevisiae, as no CFUs were observed. In fact, the higher the CuNP loading, the lower the number of CFUs reported, demonstrating a biostatic effect. Therefore, despite a possibly weaker antibacterial activity when compared to AgNPs, CuNPs are effective agent against a wide range of bacteria and fungi and have many potential applications, including the prevention of microbial surface colonization.

Titanium. Titanium dioxide (TiO2) forms active oxygen species when exposed to ultraviolet light, a process called photocatalysis. These oxygen species, including hydrogen peroxide and hydroxyl radicals, obliterate bacterial cell membranes resulting in cell death. This antimicrobial property has been utilized in water and air purification and recently has been investigated against pathogenic and opportunistic microorganisms. TiO2 nanoparticle-infused thin film composite (TFC) membranes were shown to reduce E. coli biofilm formation by disrupting the bacterial membrane and thus inhibiting bacterial attachment to the membrane surface. TiO2 has also been combined with silver to create TiO2-Ag nanoparticles (TiO2-AgNPs) that were tested against gram-negative bacteria, gram-positive bacteria and various fungi that are responsible for opportunistic infection and colonization of medical devices. Although AgNPs were found to be more effective than TiO2 and TiO2-AgNPs against the bacterial strains tested (E. coli, A. baumanii, P. aeruginosa, B. subtilis, M. smegmatic, M. bovis and S. aureus), TiO2-AgNPs demonstrated better antifungal activity against C. albicans and Aspergillus when compared to AgNPs alone and conventional antifungals such as fluconazole. Based on these results, it might be possible to combine metal nanoparticles to augment the antimicrobial activity of each alone.

Magnesium. Halogens such as chlorine, bromine and fluoride are well known for their bactericidal capabilities, but their significant toxicity hinders their direct use. The antimicrobial activity of halogens is mediated by the formation of covalent metal-halogen complexes that interact with and inhibit specific cellular enzymes. Other mechanisms may also contribute, as Mg-halogen nanoparticle have also been shown to damage the microbial cell envelope and cause leakage of intracellular contents—a process that may be mediated by lipid peroxidation due to reactive oxygen species. Of the various metals, magnesium oxide (MgO) is unique in its ability to adsorb and retain halogens, and this capacity is increased up to five times in nanoparticle formulations (MgO-NPs). Combination with MgO-NPs not only increases the antimicrobial capability of the respective halogen, but also converts the halogen into an easy to handle powder form. This enhanced antimicrobial activity was demonstrated when nanoparticulate formulations of MgO, chloride and bromide were tested against E. coli, B. megaterium and B. subtilis endospores. E. coli and B. megaterium were extremely susceptible to the MgO-halogen nanoparticles, as both species were completely killed (100%) in as little as 20 min. The B. subtilis endospores were more resistant, with only 36% of bacteria killed in 20 min. The antibacterial effects of the nanoparticulate formulations were much stronger than the halogens alone, which resulted in 68% killing in 20 min. Similar results were found using magnesium fluoride nanoparticles (MgF2-NPs), which reduced the growth of E. coli and S. aureus in a dose-dependent manner. These nanoparticles also inhibited biofilm formation of both organisms.

Zinc. Another metal oxide of interest is zinc oxide (ZnO), a compound that is approved by the FDA as a result of its antimicrobial properties and safety profile. ZnO nanoparticles (ZnO-NPs) have received considerable attention by the food industry given their demonstrated efficacy against such food-borne pathogens such as E. coli O157:H7, Listeria monocytogenes and Salmonella spp. ZnO-NPs have been shown to inhibit the growth of E. coli
O157:H7 in a dose-dependent manner, with increasing inhibitory effects as the concentration of ZnO rises. ZnO quantum dots have demonstrated several log reductions of growth of L. monocytogenes and Salmonella. These antimicrobial properties are mediated by the strong adherence of ZnO-NPs to bacterial cell membranes, which results in destruction of membrane lipids and proteins, altered membrane permeability and leakage of intracellular contents, much like the mechanism of other metal oxide nanoparticles. In addition, ZnO-NPs are thought to be strong inducers of reactive oxygen species that are harmful to bacterial cells.26

Nitric oxide-releasing nanoparticles. Nitric oxide (NO) has many roles in the human body that encompass virtually every physiological system, including host defense.27 When stimulated, phagocytic cells such as macrophages upregulate the production and release of NO through the transcription of inducible nitric oxide synthase (iNOS).28 NO is then able to exert its antimicrobial effects through several mechanisms, including direct microbial DNA damage through the generation of peroxynitrite as well as interference of cellular respiration by inactivation of zinc metalloproteins. In addition, NO can stimulate several innate antimicrobial pathways, enhancing the host’s own immune response.27,28 Given these antimicrobial properties and the ability of NO to accelerate wound healing,29 there has been tremendous interest in the development of NO donors and delivery systems.30 One such delivery system capitalizes on the benefits of nanotechnology. Utilizing NO-releasing nanoparticles (NO-NPs) housing NO within a dry matrix, the system allows release of gaseous NO free radicals only upon exposure to moisture.7,30 This delivery system is ideal for the topical treatment of wounds and infections, as NO can be easily stored and applied to the skin, and provides sustained delivery to the affected areas over a prolonged period of time.28 These NO-NPs have been tested against a variety of pathogens including S. aureus30,31 and Acinetobacter32 with considerable efficacy. In a murine wound model, MRSA-infected full thickness wounds treated with NO-NPs demonstrated accelerated wound closure and less bacterial burden when compared to wounds that were untreated or treated with control nanoparticles (no nitric oxide release).29 Similar results were found when NO-NPs were either injected subcutaneous or applied topically into induced MRSA abscesses in mice.31 Given the simplicity of their topical application, acceleration of wound healing and excellent antimicrobial properties, the potential for NO-NPs is vast.

Drug-infused nanoparticles. Intracellular infections, including those caused by facultative intracellular organisms such as Salmonella, Listeria and M. tuberculosis, are difficult to eliminate as a result of several evolutionary mechanisms. These include: escape from the phagosome, inhibition of phagosome and lysosome fusion, resistance to lysosomal enzymes, and the ability to lay dormant, all of which lead to downregulation of many drug targets. In addition, antibiotics are limited by their poor intracellular penetration and activity, further favoring microbial survival.33 In order to enhance the delivery and efficacy of antibiotics, nanoparticles and liposomes have been investigated as potential drug carriers because of their ability to be endocytosed and released into phagocytic cells carrying intracellular pathogens.33,34 Many studies report the use of nanosized vehicles to deliver antibiotics, including β-lactams such as penicillin,35 ampicillin33,36,37 and cephalosporins,33,35 as well as macrolides (azithromycin),38 aminoglycosides39 and fluoroquinolones33,39 and enhance microbial killing. For example, ampicillin-encapsulated liposomes were found to be more effective than free ampicillin against systemic Salmonella infection, substantiated by increased survival of mice in the liposome-treated group.36 Liposomal delivery has also been shown to enhance topically applied antibiotics. Liposome-encapsulated tobramycin applied topically to a rat wound model infected with P. aeruginosa resulted in reduced tissue bacterial counts, as well as sustained local tobramycin levels when compared to free tobramycin.40 In addition to targeting antibiotics, antifungals such as amphotericin B have been encapsulated into nanoparticles, leading to enhanced efficacy against molds and yeasts.34

Despite its potential, there are various limitations of nanocarriers, which include size, charge, purity, solubility of contents, stability, antigenicity and biocompatibility. Lower tolerance for variability in these parameters can lead to increased costs of synthesis and manufacture. The specificity of delivery of nanocarriers to target tissues can be passive or active. In passive targeting, nano-carriers rely on the size of apertures in neoangiogenic vessels found in tumors or fenestrations in vessels at sites of inflammation. Such targeting depends on enhanced plasma permeability and retention. In active targeting, nano-carriers require receptor-ligand interactions: In the absence of fenestrae or specific receptors, these mechanisms do not succeed. Activation of nano-carrier drug delivery by cellular activity, such as pH change or oxidative burst, can aid targeted therapy in these settings. Finally, extrinsic modes of targeting and activation, such as magnetic guidance and radio frequency mediated drug release, may be used for effecting localized delivery of nano-drugs.

Immunomodulatory Effects of Nanotechnology-Based Drug Delivery Systems

The immune system acts as the body’s major defense against foreign pathogens, and is comprised of the innate and adaptive systems. The innate or nonspecific, immune system recognizes foreign pathogens once they have breached the body’s physical barriers by recognizing microbial characteristics called pathogen-associated molecular patterns (PAMPs).41 PAMPs are recognized by pattern-recognition receptors (PRPs) that reside on a variety of host cells, initiating antigen uptake by antigen presenting cells (APCs). APCs subsequently present the antigen to cells of the adaptive or specific, immune system for the induction of specific memory T and B cells, which lead to the downstream activation of CD8 and CD4 lymphocytes.6,41 This entire process is carefully coordinated by the interactions of various cytokines, which often dictate the type of response that is generated for a given pathogen. For example, interferon-gamma (IFNγ) promotes a T helper type-1 (Th1) response that mediates antibody-independent immune responses, while interleukin-4 (IL-4) and IL-5 result in
a Th2 response, essential for antibody production. Thus the harmonized interactions of APCs, T cells, B cells and inflammatory cytokines are imperative to an effective immune system.

Vaccines are largely responsible for the reduction in mortalities due to infectious diseases, as they initiate a powerful immune response that results in lasting and protective immunity. To be effective, a vaccine must induce both an innate and adaptive immune response in a manner that is safe and beneficial to the patient. There are various existing vaccines that display different degrees of immunogenicity and safety, and include live-attenuated, killed or subunit vaccines. Although widely used, live-attenuated vaccines are unstable and have the potential to revert to a more virulent form within the host. Killed whole-virus and subunit vaccines do not carry this risk, but are less immunogenic than attenuated vaccines, requiring repeat dosing which drives cost and lowers vaccine availability. In addition, most vaccinations require trained personnel for administration since an injection is needed. Due to the increased cost and difficulty with patient compliance associated with injectable vaccine, mucosal administration is becoming a favored route due to its non-invasive nature and ease of self-administration. However, there are challenges in the development of mucosal vaccines, as the antigen has to pass several barriers before reaching an APC and must induce an immune response that is strong enough to elicit systemic immunity. For intranasal or inhalational routes, the vaccine components must be small enough to reach deep alveoli (≤5 μm) and avoid clearance during exhalation (<500 nm). As a result of their size, foreign composition and numerous carrying capabilities, nanoparticles have the ability to modulate various aspects of the immune response and may improve the efficiency of mucosal and injected vaccines because of increased exposure time and uptake of antigens by APCs, improved immunogenicity of viral and bacterial components and modulation of the cytokine response. Numerous synthetic nanoparticle vaccines and immunostimulatory adjuvants have been created and include polymeric and non-polymeric based particles. Epicutaneous delivery of vaccine may elicit a more robust immune response and may require less antigen as well.

**Nanotechnology-based vaccines and immunostimulatory adjuvants.** Synthetic polymers. Polymeric-based nanoparticles can act as carriers for a wide range of materials including protein or DNA vaccines. The characteristics of the nanoparticle in vivo is largely dependent on the type of polymer used, and therefore may be manipulated to fit the requirements of a particular vaccine. For example, to overcome compartmental differences of the digestive tract for oral vaccination, poly (ε-caprolactone) (PCL) polymers were used to create multi-component particles. These particles are made up of nanoparticle-encapsulated DNA surrounded by a PCL microparticle, known as the nanoparticle-in-microsphere hybrid oral delivery system (NiMOS). The inner part of the NiMOS is composed of gelatin nanoparticles that are susceptible to proteolytic degradation, making them vulnerable to gastric contents. The outer PCL layer is resistant to proteases but is degraded by lipases. This layer therefore protects the inner particles during transit through the stomach until reaching the small intestine, where lipases degrade PCL, allowing for release of the inner gelatin nanoparticle.

Although synthetic polymers facilitate vaccine delivery, they do not seem to be immunostimulatory on their own. Poly(lactide-co-glycolide) (PLGA) particles, for example, are efficiently taken up by macrophages and other APCs in vitro and in vivo. However, they do not lead to a significant pro-inflammatory cytokine response indicative of macrophage activation (IL-2, IL-6, IL-12 and TNFα), rather having stimulatory profiles similar to that of saline. However, when encapsulated with plasmid DNA, PLGA-based nanoparticles induced a stronger and more sustained serum IgG response after intranasal administration compared to naked DNA. It is thought that this response is due to the stability of the PLGA nanoparticles during delivery across nasal mucosa, allowing for internalization of the plasmid DNA by appropriate cells. Thus, synthetic polymers cannot be used as immunostimulatory adjuvants alone, but they can enhance immunization via delivery of antigens across mucosal barriers.

Polyethylene glycol methyl ether (PMMA) nanoparticles have demonstrated adjuvant immunostimulatory properties. PMMA NP adjuvants induce a 100-fold increased antibody titer response to live HIV2 virus vaccine in murine models. PMMA NP dendrimers have been shown in vivo to stimulate enhanced IgG and IgM anti-ovalbumin antibody production. Carboxyfullerene nanoparticles augment immunity by stimulating neutrophils to destroy bacteria in a mouse model of S. pyogenes. Nanoparticles modified with Toll-like receptor agonists have been shown to enhance immune responsiveness. For example, PLGA NP modified with tetanus toxoid and MUC1 lipopeptide (a TLR ligand) induced enhanced T cell responsiveness in vitro when compared to PLGA NP combined with tetanus toxoid or MUC1 lipopeptide alone.

Aptamers are polymeric oligonucleotides (either DNA or RNA) folded in a 3-dimensional configuration which are then selected for high affinity binding to a target. Targets for aptamers include proteins, peptides and small molecules such as drugs, vitamins and inorganic compounds. Aptamers can be selected from random nucleic acid libraries, and can be prepared to high degrees of purity, stability and selectivity. Aptamers have been used to create compounds with antimicrobial activity, including the ability to inhibit HIV reverse transcriptase, vaccinia virus replication, and in vitro models of β-lactamase resistance in Gram-positive and Gram-negative bacteria. Successful PEGylated delivery of an aptamer inhibitor of VEG-F has been used in clinical trials of macular degeneration.

**Nanoemulsions.** Nanoemulsions (NEs) encapsulate lipophilic or hydrophilic substances in a dispersed phase and are manufactured as water-in-oil (W/O) or oil-in-water (O/W) systems. These carrier systems can be used in the development of mucosal vaccines, as they can be endocytosed by cells on the mucosal surface (epithelial or M cells) and subsequently delivered to APCs. In addition, NEs are themselves immunostimulatory and can be used to boost the immune response of a mucosal vaccine. The current hepatitis B vaccine requires an intramuscular (IM) injection and consists of recombinant hepatitis B surface antigen (HBsAg) formulated with an aluminum salt (alum) adjuvant.
This adjuvant elicits a Th2 immune response associated with an ineffective CD8 response to virally-infected cells. Moreover, adverse effects to the alum have also been reported and include nodules and erythema at the site of injection. In contrast, recombinant HBsAg nanoemulsion (HBsAg-NE)-based vaccines induced a Th1 response and effective cellular immunity in animals, and did not produce local inflammation after injection. When formulated into an intranasal vaccine, HBsAg-NE produced high levels of anti-HBsAg serum IgG antibodies that were comparable to IM HBsAg-NE and HBsAg-alum. In addition, intranasal HBsAg-NE induced a Th1 cytokine profile and significant levels of mucosal IgA antibodies. NE-based vaccine have also been shown to be effective mucosal adjuvants for inactivated influenza virus and vaccinia viruses among other pathogens.

**Immune-stimulating complexes.** Immune-stimulating complexes (ISCOMs) are nanosized spherical micelles that act as carriers and immunostimululatory adjuvants due to their saponin-derived components such as Quil A, which originates from the bark of the Quillaja tree. As carriers, ISCOMs are efficiently taken up by APCs as a result of their particulate nature. In addition, even in the absence of antigen, they are able to activate and upregulate expression of MHC I and II on APCs, initiating an adaptive immune response. This results from the ability of Quil A to induce pro-inflammatory cytokines such as IL1, IL6, IL8 and IFNγ. Furthermore, saponin-derived components are capable of producing a Th1-type response, but this may be modified by the type of antigen or adjuvant carried. For example, ISCOMs carrying Leishmania antigen were shown to induce high levels of IL4, indicative of a Th2 polarized response. Numerous antigens have been incorporated into ISCOMs and have been shown to induce protective immune responses, including influenza, hepatitis B, herpes virus and bacteria such as Helicobacter pylori and Corynebacterium.

Cytidine-phosphate-guanosine (CpG) motifs. Bacterial DNA sequences, specifically oligodeoxynucleotides containing unmethylated CpG motifs, have been found to be immunostimulatory and therefore have been used as vaccine adjuvants. CpG motifs are recognized by APCs through activation of Toll-like receptor 9 (TLR-9). This activates secretion of IL12 and expression of MHC and co-stimulatory molecules by monocytes and dendritic cells, subsequently inducing a Th1 immune response and generation of IgG antibodies. When incorporated into nanoparticle formulations, CpG motifs induce a strong and sustained immune response. For example, a multiple nanoemulsion system (W/O/W) formulated with CpG and inactivated influenza virus (PELC/CpG) was found to induce a higher antigen-specific serum antibody response after only one dose when compared to two doses of a similar but non-adjuvanted vaccine. Furthermore, this PELC/CpG induced a higher antibody response than was obtained from vaccine adjuvanted with alum.

**Chitosan.** As discussed previously, chitosan can be used as a carrier system, and this property allows for improved delivery of vaccine antigens across mucous membranes. It also acts as an immune adjuvant as a result of its ability to promote antigen uptake and cytokine production. As a result, when incorporated with influenza surface glycoproteins HA and NA, chitosan nanoparticles induced a greater serum (IgG) and mucosal (IgA) antibody response than purified surface antigens alone after intranasal administration. Similar results were found when Streptococcus equi protein antigen and cholera toxin B immune adjuvant were encapsulated into PCL nanoparticles modified with chitosan, spermine or oleic acid adsorption enhancers, as chitosan-based particles induced a high serum IgG and secretory IgA response. The high IgA response is thought to result from chitosan’s strong interactions with sialic acid residues in mucin, which increases vaccine residence time and improves membrane transport by inducing the transient opening of mucosal tight junctions. Chitosan formulations have also shown promise as immunostimulatory adjuvants in intradermal vaccinations. N-trimethyl chitosan (TMC) nanoparticles were formulated with ovalbumin (OVA) or diphtheria toxoid (DT) and injected intradermally into mice. TMC nanoparticles were taken up by dendritic cells (DC) in the skin and induced the expression of CD83, CD86 and MHC-II, signifying DC maturation. Once internalized, the antigens (OVA and DT) were released from chitosan by lysosomal degradation, and subsequently processed and presented to T cells, inducing a Th2 antibody response. With both antigens, TMC induced higher IgG titers than either antigen alone, substantiating its role as an immunostimulatory adjuvant in intradermal vaccination.

**Metallic nanoparticles.** When used as drug- or gene-delivery vehicles, nanoparticles must hide their contents during transport to their intended target in order to avoid immune system activation and cell toxicity. This property has been shown in metallic nanoparticles, substantiating their use as carrier systems. For instance, foreign nucleic acids are known to activate the innate immune response, complicating gene delivery. However, when conjugated with gold nanoparticles, the immune response is dampened. Compared to lipid-complexed DNA, the macrophage response to a polyvalent oligonucleotide-modified gold nanoparticle (DNA-Au NP) conjugate was 25-fold less, as evidenced by decreased levels of IFNβ despite higher amounts of DNA internalization. It is thought that the DNA-Au NP conjugates allow a high oligonucleotide surface density, which reduces the ability of cellular DNA binding proteins to recognize nucleic acids on the nanoparticle. This was further validated by an increased innate immune response with decreasing DNA surface density. The immune response is also highly dependent on the antigen that is being carried, particularly the peptide sequence of the conjugate. For example, gold nanoparticles (AuNPs) alone did not affect macrophage proliferation or cytokine response, while AuNP conjugates blocked macrophage proliferation and induced cytokine production depending on the specific peptide sequence of the conjugate. Even more interesting, although all AuNP conjugates induced TNFα and IL1β, only AuNP-CLPFFD-NH₂ induced a strong IL6 response.

**Conclusions**

Nanotechnology allows for the creation of unique carrier systems that enhance molecular interactions, thus allowing nanoparticles to facilitate the body’s response to foreign pathogens. This includes...
surpassing microbial resistance mechanisms and improving the innate and adaptive immune response. Incorporating conventional antimicrobials or other materials into nanoparticle platforms also allows for targeted drug delivery and minimizes drug resistance. In the case of antimicrobial agents, the large surface-to-volume ratio of nanoparticles increases drug penetration by disrupting the microbial cell wall or cytoplasmic membrane. When engineered to carry foreign antigens, nanoparticles may also act as immuno-stimulatory adjuvants as a result of increased uptake by antigen presenting cells and subsequent Th1 or Th2 immune response.

Importantly, future studies will be needed to explore potential deleterious effects of these agents. Even today, there is growing concern regarding toxicity as a result of specific materials used in some particles (i.e., heavy metals) and their ability to penetrate vital organs, although the evidence regarding human toxicity is inconclusive to date. Regardless, the future of nanotechnology in the antimicrobial arena is not only bright, but a requisite to successfully combat the medical crisis that has resulted from the continued emergence of resistant infectious organisms.

Acknowledgments

The authors acknowledge Dr. Joshua Nosanchuk for his insight and his passion for infectious diseases.
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