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Silver nanoparticles interactions with the immune system: implications for health and disease

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1. Introduction

Our immune system constantly interacts with our internal environment, protects us from our external environment and provides the inherent knowledge to sense the difference between friend and foe with important implications in human health and disease (Pozo, 2008). For these reasons, it is important to identify functional alteration of key immune responses as the number of silver nano-enabled products grows while the current data strongly suggest that other related nanomaterials, such as polymer nanoparticles, fullerenes, dendrimers and gold nanoparticles, interact with the immune system.

In the present chapter, we will focus on the effects of engineered silver nanoparticles on the initiation and regulation of the immune response. Particular attention will be paid on the potential clinical usefulness of silver nanoparticles in the context of its effects on the production of key immunological mediators, as well as its significance on bacterial and viral infections. By a critical analysis of the current state of knowledge, the chapter will help to reduce the serious lack of information and controversial issues concerning the biological effects of silver nanoparticles on the immune system.

2. The immune system

Studies on the biological consequences of new nanomaterials suited for biomedical applications are of importance, particularly those related to the immune system. Therefore, the interactions of silver nanoparticles with the immune system and its potential effects and implications are key questions for nanomaterials that are intended for biomedical applications or extensive industrial manufacturing such as those considered in this chapter. For this reason, before explaining the variety of interactions, effects and implications, we

will start with a brief introduction about the functions of the immune system and its cellular components.

The immune system is a dynamic network of cells, tissues, and organs that work coordinated to defend the body against attacks by "foreign" invaders and protects against disease by identifying "self" and "non-self" (for example virus, fungus, bacterium) cells and tissues (Christensen & Thomsen, 2009).

To deal with antigens, the system uses specialized cells to recognize infiltrators and eliminate them. Detection is complicated as pathogens can evolve rapidly, producing adaptations that avoid the immune system and allow the pathogens to successfully infect their hosts (Christensen & Thomsen, 2009). For cellular communication there is a category of signaling molecules called cytokines. They are small secreted proteins which are critical to the development and functioning of both the innate and adaptive immune response, although they are not limited just to the immune system. They act by binding to specific membrane receptors, which produce cascades of intracellular signalling to alter cell functions. Cytokines have been classified as, lymphokines (cytokines made by lymphocytes), monokines (cytokines made by monocytes), chemokines (cytokines with chemotactic activities), and interleukins (cytokines made by one leukocyte to act on other leukocytes). The actions of cytokines may be grouped as, autocrine action (the cytokine acts on the cell that secretes it), paracrine action (the target is restricted to nearby cells), and endocrine action (the cytokine diffuses to distant regions of the body).

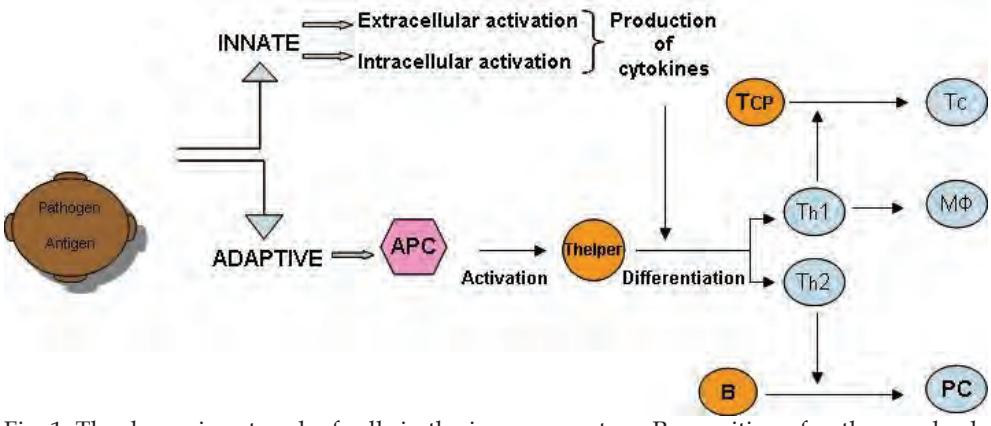

Fig. 1. The dynamic network of cells in the immune system. Recognition of pathogens leads the activation of T helper cells (Th). This process involves adaptive events that occur on the surface of the antigen presenting cells (APC). They display a foreign antigen complex with MHC on its surface recognized by T-cells using their T-cell receptor (TCR). The immune system contains three types of APC macrophages, dendritic cells and B cells. APC collaborate with the innate response by cytokine communication, which steer the differentiation of Th cells into Th1 or Th2 subsets. These T cells activate other cells of the immune system such as cytotoxic T cells (Tc), and macrophages (MΦ). Silver nanoparticles can interact with the immune system and carry out different functions like binding and reacting with cells or proteins for drug delivery, detection, diagnosis and therapy (targeted and non-targeted).

2.1 Adaptive immunity

As part of this complex immune response, the human immune system adapts over time to recognize specific pathogens more efficiently. This adaptation process is referred to as adaptive immunity and creates immunological memory from a primary response to a specific pathogen, provides an enhanced response to secondary encounters with that same, specific pathogen.

2.2 Innate immunity

The innate immune system comprises the cells and mechanisms that defend the host from infection by other organisms, in a non-specific manner (Castellheim *et al.*, 2009). This means that cells of the innate system recognize and respond to pathogens in a generic way, but unlike the adaptive immune system, does not confer long-lasting or protective immunity to the host. Innate immune systems provide immediate defense against infection (Rasmussen *et al.*, 2009).

3. Silver nanoparticles

In nanotechnology, a nanoparticle is defined as a material with dimensions and tolerance limits of 0.1-100nm that behaves as a whole unit in terms of its transport, properties and unique characteristics.

Metallic nanoparticles have unique optical, electrical and biological properties that have attracted significant attention due to their potential use in many applications, such as catalysis, biosensing, drug delivery and nanodevice fabrication. Capped silver nanoparticles (AgNPs) have many biomedical applications due to its excellent biocompatibility and antibacterial properties. It has been reported that silver nanoparticles interact with virus, bacteria, and the immune system, being the reason why in this chapter we will explain how the size, shape and composition of silver nanoparticles can have a significant effect on their efficacy and have to be kept in mind when using bioconjugates.

3.1 Interaction of silver nanoparticles with key signaling pathways and soluble mediators

Activation of the innate immune system is mediated by pattern recognition receptors (PRRs) on particular immunocompetent cells that recognize pathogen-associated molecular patterns. The best characterized signaling PRRs to date are the Toll-like receptors (TLRs) present in plants, invertebrates and vertebrates that represent a primitive host defense mechanism against bacteria, fungi and viruses. Toll-like receptors (TLRs) play an important role in innate immunity. Individual TLRs recognise microbial components that are conserved among pathogens, such recognition initiates necessary inflammatory immune responses and induces subsequent activation of adaptive immunity (Uematsu & Akira, 2006). In conclusion TLRs are a type of pattern recognition receptors (PRRs) which recognize molecules broadly shared by pathogens but distinguishable from host molecules. TLRs can be divided into two groups according to their cellular localization: TLRs 1, 2, 4, 5 and 6 are mainly located on the cell surface and primarily recognize bacterial components, while TLRs 3, 7, 8 and 9 are mostly found in the endocytic compartments and mainly recognize viral products (Akira *et al.*, 2006).

Toll-like receptors (TLR) and their ligands are one of the main players in the initiation of innate immunity which precedes, and is required, for the establishment of adaptive immunity. Manipulating the immune response by using TLR agonists or antagonists might be of therapeutic and/or prophylactic value (Makkouk & Abdelnoor, 2009). Exogenous signals are provided by TLRs mechanisms which affect the initiation, maintenance and progression of inflammatory diseases. Moreover, reagents that enhance TLR signalling pathways can be powerful adjuvants for fighting pathogens or cancer. By contrast TLR antagonists or signalling inhibitors could block the activation of TLRs by neutralizing the ligands, blocking the receptors or preventing signalling, which can also have other beneficial therapeutic effects in autoimmune diseases and sepsis (Zuany-Amorim *et al.*, 2002).
This opens up new avenues in the world of adjuvants and illustrates the basic requirements for the design of NP conjugates that efficiently reach their target.
It has recently been studied whether AgNPs with a narrow size distribution and protected with a monolayer of adsorbed tiopronin (Ag@tiopronin) had any functional impact on specific TLR stimulation of Interleukin-6 (IL-6) secretion in Raw 264.7 macrophages, a murine monocyte/macrophage cell line (Castillo *et al.*, 2008), and the effects of silver nanoparticles on cytokine expression by peripheral blood mononuclear cells (PBMC) (Shin *et al.*, 2007), human mesenchymal stem cells (hMSCs) (Greulich *et al.*, 2009), and J774 A1 macrophages (Yen *et al.*, 2009).

Nanoparticles	Cell type	Concentration	Size (nm)	Cytokines	Ref
Ag@tiopronin	Raw 264.7 macrophages	10 μg/mL	~5	IL-6	Castillo et al (2008)
AgNPs	PBMC	1, 3, 5, 10, 20 μg/mL	~1.5	IL-5 INF- γ TNF-α	Shin et al (2007)
AgNPs	J774 A1 macrophages	1 μg/mL	Small: 2-4 Medium: 5-7 Large: 20-40	IL-1 IL-6 TNF-α	Greulich et al (2009)
AgNPs	hMSCs	0.05, 0.5, 1, 2.5, 5, 50 μg/mL	100	IL-6 IL-8 IL-11	Yen et al (2009)

Table 1. The effect of silver nanoparticles on the production of cytokines. Silver NPs were found to strongly inhibit cytokine production by Raw 264.7 macrophages, PBMC, hMSCs and more weakly by J774 A1 macrophages.

The results demonstrated that Ag@tiopronin do not show proinflammatory effects on macrophages and that interestingly, Ag@tiopronins differentially inhibits the IL-6 secretion

mediated by specific TLRs located in the cell surface or in the endocytic compartments (Castillo *et al.*, 2008).

Moreover, the effects of silver nanoparticles on the production of cytokines by PBMC, hMSCs, were found to strongly inhibit cytokine production, for example, of INF- γ, IL-6, IL-8, IL-11, TNF-α and more weakly IL-5. In the case of J774 A1 macrophages the expression levels of IL-1 and IL-6 were similar to controls.
The biological effects of nanoparticles were analyzed by enzyme-linked immunosorbent assay (ELISA) in the case of PBMCs stimulated by phytohaemagglutinin (PHA) in the presence of NPs (Shin *et al.*, 2007) and hMSCs stimulated previously by lipopolysaccaride (LPS) (Greulich *et al.*, 2009). In the case of non-stimulated J774 A1 macrophages, the cytokine mRNA expression levels were analyzed in cells treated with NPs and were compared to the resting state, measured by RT-PCR (Yen *et al.*, 2009).
In contrast there are other nanoparticles such as SiO_2 nanoparticles (Lucarelli *et al.*, 2004), gold nanoparticles (AuNPs)(Yen *et al.*, 2009), or very small size proteoliposomes (VSSP) (Venier *et al.*, 2007), that are dramatically pro-inflamatory.
However, in these studies, nanoparticles had a very heterogeneous size (from 1.5 to 100 nm) and concentration (from 0.05 to 50 μg/mL) (see Table 1), so it is difficult to establish whether the effect is related to the chemical nature, the size or the concentration. In any case, in these different experiments, exposures to concentrations lower than 3 ppm did not produce a significant decrease of cytokine production. These effects can be considered for future medical applications, but we have to keep in mind the characteristics previously mentioned, as others like the easily uptake into cells or tissues and not so easy metabolism.

3.2 Interaction of silver nanoparticles with virus

The interaction of silver nanoparticles with viruses is a largely unexplored field. A virus is a sub-microscopic infectious agent that can only multiply in living cells of animals, plants, or bacteria. Viruses are about 1/100th the size of bacteria and consist of a single- or double-stranded nucleic acid (DNA or RNA) surrounded by a protein shell called a capsid. Some viruses have an outer envelope composed of lipids and proteins. The viral capsid proteins bind to the host cellular surface specific receptors. This attachment can induce the viral envelope protein to undergo changes that results in the fusion of viral and cellular membranes and may lead to an infection. For this reason there is a high interest studying possible mechanisms of binding NPs to the viral capsid and inhibit the later fusion.
Recently, it has been suggested that nanoparticles bind with a viral envelope glycoprotein and inhibit the virus by binding to the disulfide bond regions
of the CD4 binding domain within the gp120 glycoprotein, as demonstrated in vitro (Elechiguerra *et al.*, 2005). Silver nanoparticles undergo a size-dependent interaction with HIV-1, nanoparticles ranging from 1 to 10 nm attached to the virus, and their surface chemistry can modify their interactions with viruses, tested with silver NPs that had three different surface chemistries: foamy carbon, poly (N-vinyl-2-pyrrolidone) (PVP), and bovine serum albumin (BSA). Differences have been observed in HIV-1 inhibition and can be justified because BSA and PVP are directly bounded to the nanoparticle surface and are totally encapsulated, while the foamy carbon silver nanoparticles have fundamentally a free surface area, which exhibit higher inhibitory effect and cytotoxicity as they are able to have stronger interactions (Elechiguerra *et al.*, 2005).

Within this unexplored area there are also other studies that analyse the interaction of silver nanoparticles with hepatitis B virus. The effects of silver NPs on hepatitis B virus (HBV) have been reported using an infection model HepAD38 human hepatoma cell line (Lu *et al.*, 2008). The binding affinity of NPs with different sizes (10 and 50 nm) for HBV DNA and extracellular virions resulted very high and could also inhibit the production of HBV RNA and extracellular virions in vitro, which was determined using a UV-vis absorption titration assay(Lu *et al.*, 2008). It will need further investigation to find whether this binding activity prevents HBV virions from entering into host cells or not. In vivo studies with silver NPs are necessary to design therapeutics and vaccines that can specifically target viruses in order to increase therapeutic benefit and minimize adverse effects.

3.3 Interaction of silver nanoparticles with bacteria

Bacteria are prokaryotic, microscopic, single-celled organisms that lack membrane bound organelle in the cytoplasm. They can inhabit all kinds of environments and exist either as independent (free-living) organisms or as parasites.

Silver has been used for at least six millennia in order to prevent microbial infections. It has been used to treat a wide variety of infections and has been effective against almost all organisms tested. Silver compounds were major weapons against wound infection in World War I until the advent of antibiotics and between 1900 and 1940, tens of thousands of patients consumed colloidal silver, and several million of doses were given intravenously. But it was shown that high doses of silver, when administered intravenously could cause convulsions or even death, and that oral administration of high doses could cause gastrointestinal disturbances (Alexander, 2009). For this reason the biomedical applications of silver can be effective by the use of biologically synthesized NPs, which minimize the factors such as toxicity and cost, and are found to be exceptionally stable and by virtue of extremely small size silver NPs exhibit unusual physicochemical properties and biological activities. Due to the large surface area (generally the diameter is smaller than 100nm and contains 20–15,000 silver atoms) for reaction of the NPs, the dose of silver used in medical applications can be reduced.

The mechanisms of action and binding of silver nanoparticles to microbes remain unclear but it is known that silver binds to the bacterial cell wall and cell membrane and inhibits the respiration process (Klasen, 2000) by which the chemical energy of molecules is released and partially captured in the form of ATP. Silver nanoparticles interact with sulfur-containing proteins of the bacterial membrane as well as with the phosphorus containing compounds like DNA to inhibit replication (Silver *et al.*, 2006). Bactericidal effect of silver has also been attributed to inactivation of the enzyme phosphomannose isomerase (Bhattacharya & Mukherjee, 2008), that catalyzes the conversion of mannose-6-phosphate to fructose-6-phosphate which is an important intermediate of glycolysis, the most common pathway in bacteria for sugar catabolism.

The antimicrobial activity of silver nanoparticles has been investigated against yeast, gram negative and positive bacteria (Kim *et al.*, 2007) (Sondi & Salopek-Sondi, 2004; Yu, 2007). When silver nanoparticles were tested in yeast and *Escherichia coli* (Gram -), bacterial growth was inhibited (Sondi & Salopek-Sondi, 2004), but the inhibitory effect in *Staphylococcus aureus* (Gram +) was mild (Kim *et al.*, 2007). Therefore, this suggests that the antimicrobial effects of silver nanoaparticles can be associated with different characteristics of the membrane structure, in order to the considerable differences between the membrane

structures of Gram+ and Gram-. These differences mainly rely on the peptydoglycan layer thickness, the rigidity and extended cross linking that makes the penetration of nanoparticles very difficult.

Recently, due to the emergence of antibiotic-resistant bacteria and the use limitations of antibiotics that can cause serious diseases and is an important public health problem (Furuya & Lowy, 2006), the synergetic effect of silver nanoparticles with antibiotics has been studied combinating silver nanoparticles with different antibiotics like ampicillin, kanamycin, erythromycin and chloramphenicol against gram positive and gram negative bacteria. The antibacterial activities of these antibiotics increase in the presence of silver nanoparticles against gram positive and gram negative bacteria determined by the disk diffusion method. Different diameters of inhibition zones have been shown around the different antibiotic disk with or without AgNPs. The combination effect of nanosilver and ampicillin has more potential compared to the other antibiotics and may be caused by both, the cell wall lysis action of the ampicillin and the DNA binding action of the silver nanoparticles (Fayaz *et al.*, 2009). The antibiotic molecules contain many active groups such as hydroxyl and amido groups, which reacts easily with silver nanoparticles by chelation, for this reason, the synergistic effect may be caused by the bonding reaction with antibiotic and silver nanoparticles.

The bactericidal effects of silver nanoparticles are part of an extensive research field due to its potential translation for biomedical applications such as, wound-healing (Tian *et al.*, 2007; Silver *et al.*, 2006), clothes, coating for medical devices (Roe *et al.*, 2008), antimicrobial gel (Jain *et al.*, 2009), and orthopaedic implants (Nair & Laurencin, 2008).

It is well known that the use of central venous catheters is associated with bactericidal line infections, which is a usual problem (Stevens *et al.*, 2009). Contaminated or infected catheters are a major source of nosocomial infections responsible for > 40% of all episodes of nosocomial sepsis in acute-care hospitals (Samuel & Guggenbichler, 2004). For this reason catheters coated with silver NPs are important to confer antimicrobial activity and play an essential part in the prevention of catheter-related infections.

In vivo studies have been performed to test the antimicrobial activity of catheters coated with silver NPs and it has been reported that the coating process is slowly reversible, yielding sustained release of silver for at least 10 days (Roe *et al.*, 2008). Each animal (C57B1/6J male mice) was implanted with the equivalent of a 28 cm silver coated catheters and showed no sign of toxicity, inflammation or infection at the site of catheter implantation. The released silver is active against microorganisms with no risk of systemic toxicity and safety of use in animals. This suggests that catheters coated with this method could provide local protection against infections (Roe *et al.*, 2008).

However, some questions need to be addressed and more elaborate experimental evidences will be needed to clarify such as the exact mechanism of interaction of silver nanoparticles with the bacterial cells and the killing activity of the nanoparticle surface (Rai *et al.*, 2009).

3.4 Cytotoxicity, biodistribution and clearance.

Biomedical applications of silver nanoparticles need to be cytocompatible and have the capacity to restore natural morphology of the tissue in contact without triggering immunogenecity. Therefore, the evaluations of these effects are of high importance for future medical purposes and prior to in vivo applications, in vitro methods are needed to evaluate cytocompatibility as a prerequisite. Silver nanoparticles studies work as basic tools

for evaluating nanoparticle safety in order to foster the efficient movement of AgNPs products through preclinical and clinical development.
Different assays are used to measure cell viability such as MTT assays, LDH assays and apoptosis/necrosis assays among others. MTT [3-(4,5-dimethylthiazoyl-2-yl)-2,5-diphenyltetrazoliumbromide] assays, used to demonstrate the viability of cells through the reduction of the pale yellow MTT dye to a dark blue formazan product by the activity of succinate dehydrogenase present in the mithocondria of living cells, LDH (lactate dehydrogenase) assays, that consists in measuring the release of this LDH stable cytoplasmic enzyme that is rapidly released into the cell-culture supernantant upon damage of the plasma membrane and apoptosis/necrosis assays detects changes in cell membrane permeability (Weyermann *et al.*, 2005).

As it has been reported, tiopronin silver nanoparticles (Castillo *et al.*, 2008), BSA capped Ag-Pt alloy nanoparticles (Singh *et al.*, 2009) and silver nanoparticles protected with Na^+-poly (γ -glutamic acid) (Yu, 2007) are not cytotoxic, while, by contrast bare silver nanoparticles have been found to be rather toxic (Braydich-Stolle *et al.*, 2005; Kim *et al.*, 2009; Hussain *et al.*, 2005). This supports the idea that the toxicity is associated to the presence of bare metallic nanoparticle surfaces, while particles protected by and organic layer are much less toxic except starch capped nanoparticles which present mithocondrial dysfunction , induction of reactive oxygen species (ROS), DNA damage and cell cycle arrest (AshaRani *et al.*, 2009).
Serious limitations of these studies assessing biological properties of nanoparticles exist, such as the partial characterization of the material used and its size heterogeneity. This is one of the main causes of discrepancies in the literature (See Table 2).

Nanoparticles	Concentration	Size (nm)	Cell type	Toxicity signs	Ref
AgNPs	10 µg/mL	2-40	Murine macrophages	Spread shape	Greulich et al (2009)
Starch-capped-NPs	25-400 µg/mL	6-20	IMR-90 U251	◦Mithocondrial damage ◦ Increase of ROS ◦ Reduced ATP content ◦ DNA damage ◦ Cell cycle arrest	AshaRani et al (2009)
AgNPs	10 µg/mL	15	C18-4	◦ Cell morphology changes ◦ Reduced mithocondrial function ◦Slight LDH leakage	Braydich-Stolle et al (2009)

AgNPs	0.1-10 μg/mL	5-10	HepG2	◦ Reduced mithocondrial function ◦LDH leakage ◦Increase of ROS	Kim et al (2009)
AgNPs	5-50 μg/mL	15, 100	BRL 3A	◦Reduced mithocondrial function ◦LDH leakage ◦ Depletion of GSH level ◦Increase of ROS	Hussain et al (2005)
BSA capped Ag-Pt alloy NPs	50-100 μM	10-15	HGF	None	Singh et al (2009)
Ag@tiopronin	10 μg/mL	~5	Raw 264.7 macrophages	None	Castillo et al (2008)

Table 2. In vitro cytotoxicity studies of silver NPs on different cell lines. Toxicity signs seem to be associated to the presence of bare metallic nanoparticle surfaces, while particles protected by and organic layer seem to be much less toxic.

Toxicity studies with silver NPs have been made in vivo using a zebrafish embryonic model, based on the putative similarity between the zebrafish and human genomes (Bar-Ilan *et al.*, 2009). All sizes of colloidal silver NPs caused toxicity in zebrafish embryos in a size-dependent manner for certain concentrations and time points. Exposure to 250 μM (3, 10, 50 and 100nm) of colloidal silver nanoparticles caused significantly different percentages of mortality, with 80%, 64%, 36%, and 3% respectively, by 24 hours post-fertilization; 250 μM of colloidal silver nanoparticles of all sizes caused almost 100% mortality by 120 hours post-fertilization. Lethality caused by 100 μM is significantly lower than mortality caused by 250 μM at almost all time points, although 100 μM of colloidal silver NPs causes low lethality that increases with time, the induced sublethal toxic effects are represented by high average toxicity values. Overall, there are other many different parameters that could be responsible for the adverse effects that induce nanoparticle toxicity, such as, concentration, stability, chemistry and/or functionalization (Bar-Ilan *et al.*, 2009)

The human body has several semi-open interfaces such as the respiratory tract, or gastrointestinal tract and skin for direct substance exchange with the environment and are also the principle routes of exposure to silver nanoparticles (Chen & Schluesener, 2008). At these sites, silver nanoparticles can carry out different functions like binding and reacting with proteins, deposition, clearance and translocation. If the administration comes from other portals, the nanoparticles will have a direct contact with blood components, with the cardiovascular system and are distributed throughout the body. For this reason, the need for comprehensive Absorption, Distribution, Metabolism and Excretion (ADME) studies for nanomaterials are crucial to provide examples of how physical properties affect the state of agglomeration or aggregation, surface characteristics and stability(Zolnik & Sadrieh, 2009).

However, the need for an open dialogue between industry, academia, research labs and regulatory agencies cannot be overemphasized and as such, active collaboration should be facilitated, so that safe and effective nanotechnology products are developed for clinical use to treat the complex diseases.

4. Preclinical studies and clinical trials

In terms of healing, the elucidation of pro-inflammatory and anti-inflammatoyry pathways is important for the development of strategies to defend regenerative tissue from damage caused by imbalances in cytokines, oxidants, antioxidants within the wound. Recently, information about specific subsets of inflammatory cell lineages and the cytokine network orchestrating inflammation associated with tissue repair has increased (Eming *et al.*, 2007).
It is known that silver NPs can promote wound healing and reduce scar appearance in a dose-dependent manner and that cytokines play an important role in these processes by their capacity to decrease wound inflammation and modulate fibrogenic cytokines (Tian *et al.*, 2007; Wong *et al.*, 2009). It has been shown in vivo that silver NPs act decreasing inflammation through cytokine modulation.
Two animal models have been used for these experiments, twenty-week-old male BALB/C mice were used for all thermal injury experiments and C57BLKs/J-m +ldb, db/db (genetically diabetic) and C57BLKs/J-m (nondiabetic control) male mice were used for the impaired wound-healing animal model.
For thermal injury the dorsum of each mouse was shaved from the base of the tail to the base of the neck, anesthetized and lade on a burn template. For the chronic wound model, the hair on the back of each mouse was shaved, and a piece of full-thickness skin was excised with scissors. The experiments were performed with spherical silver NPs (~14nm) at 1mM. The expression patterns of IL-6, TGF-β1, IL-10, VEGF (vascular endothelial growth factor, a polypeptide that stimulates the growth of new blood vessels), and IFN-γ were investigated by real-time RT-PCR. Expression levels of IL-6 mRNA in the wound areas treated with silver NPs were maintained at statistically significant lower levels during the healing process while TGF-β1 levels were higher in the initial period of healing but decreased at a lower level during the later phase of healing. Furthermore, IL-10, VEGF and IFN-γ expression levels stayed higher in animals treated with silver NPs relative to the no-treated ones. These results can confirm that silver NPs can modulate cytokine expression (Tian *et al.*, 2007).
At the time of injury, the production of pro-inflammatory cytokines and the expression of E-selectin, chemokines and integrin ligands on endothelial cells mediate the selective recruitment of cutaneous lymphocyte-associated antigen positiveT-cells into the wound where they recognise the antigen for which their receptor is specific and become activated. The macrophages act as antigen presenting-cells and also express the co-stimulatory molecules that are essential for the T-cell activation. After antigen binding; T-cells differentiate into subtypes, preferentially into Th1 subsets and secrete interferon-gamma.
Interferon-gamma is the major macrophage activating cytokine and enhances the effective functions of macrophages (Tsirogianni *et al.*, 2006). The functional diversity of cytokines is thought to be important in dictating different phases of immunoinflammatory responses. Th1 cytokines are mostly involved in cell-mediated immunity associated with autoimmune disorders and allograft rejection, whereas Th2 cytokines are mostly involved in mediating

allergic inflammation and chronic fibroproliferative disorders. The initial period of 4–5 weeks of infection is largely driven by the Th1 cytokine. The Th1 response in the early phase of the infection is initiated by higher numbers of IFN-γ- secreting CD4 and CD8 cells in the spleen and lymph nodes (Azouz *et al.*, 2004). For this reason to accomplish successful wound repair and tissue regeneration, the inflammatory response must be tightly regulated by silver NPs. The lack of amplification of the inflammatory cytokine cascade is important in providing a permissive environment for wound repair and tissue regeneration to proceed. These results have given an insight into the actions of silver and provided a novel therapeutic direction for wound treatment in clinical practice. Other experiments need to be focused on the effect of silver NPs on the lymphocyte subset of the Th1/Th2 profile and see how silver NPs affect their particular cytokine production.
Moreover, other studies have been developed in vivo with a rat model of ulcerative colitis treated with nanocrystalline silver (40, 4 and 0.4 mg/kg) which demonstrated to have antimicrobial and anti-inflammatory properties (Bhol & Schechter, 2007). Ulcerative colitis is a form of inflamatory bowel disease which is a chronic inflammatory condition of the gastrointestinal tract. Ulcerative colitis is a disease of the large intestine or colon, which includes characteristic ulcers, or open sores. Nanocrystalline silver (NP 32101) was administered intracolonically or orally and the study revealed that intracolonic treatment of NP 32101 at concentrations of 40 and 4 mg/kg significantly reduced colonic inflammation and that oral treatment with 40mg/kg also improved colonic lesions but was not effective at concentrations of 0.4 and 4 mg/kg. These results suggest that NP 32101 is much more effective when delivered locally to the target organ due to the increased potency with intracolonic treatment (Bhol & Schechter, 2007).

Other experiments with nanocrystalline silver cream have been made using a murine model of allergic contact dermatitis. Dermatitis was induced on the ears of BALB/c mice using dinitrofluorobenzene and later treated with 1% of nanocrystalline cream, tacrolimus ointment (topical drug used for the treatment of eczema) or a high potency steroid, applied once a day for four days. The results showed significant reductions of ear swelling, erythema and histopathological inflammation with no significant difference between treatments. The effect of topical nanocrystalline silver on the induction of apoptosis of inflammatory cells and the role of nanocrystalline silver in suppresion of inflammatory cytokines was examined by measuring the mRNA expression and protein expression of IL-12 and TNF-α. The expression of these inflammatory cytokines were significantly suppressed by nanocrystalline silver, tacrolimus and high potency steroid, but test sites treated with nanocrystalline silver showed more extensive apoptosis of inflammatory cells than the test sites treated with the other treatments (Bhol & Schechter, 2005) .

Other in vivo studies will be needed for further assessment of the NPs in various fields such as medical devices, antimicrobial systems and drug delivery.

At present clinical trials are being performed comparing central venous catheters with silver NPs versus conventional catheters. This study is currently recruiting participants. Catheters coated and/or impregnated with different antimicrobial agents have been proposed to reduce the risk of such infections. However, results obtained so far did not reach enough clinical relevance to consider these medicated catheters as a valid alternative to the

conventional ones. (Data was obtained from Clinicaltrials.gov, a service of the U.S Natoinal Institutes of Health)
Other clinical studies are being carried out about the efficacy of silver nanoparticle gel versus a common antibacterial hand gel. This study is also recruiting participants and the specific aims are to compare the immediate antimicrobial efficacy of a one-time application of silver nanoparticle gel, compare the persistent antimicrobial efficacy of a one-time application of silver nanoparticle gel (SilvaSorb®) versus an alcohol-based hand gel (Purell®) over a 10 minute time frame in producing a persistent reduction on transient bacterial counts isolated from hands seeded with S. marcescens and finally to Compare user acceptability of silver nanoparticle gel (SilvaSorb®) versus an alcohol-based hand gel (Purell®) using a self-assessment questionnaire.

5. Future research

The future research is now being shaping by a number of research groups that are actively trying to combine a variety of functions into so-called multifunctional silver nanoparticles. We can expect examples of applications that could include a metallic or semi-metallic core that responds to external energy field or which contains a delivered agent; targeting biomolecules for delivery to specific cellular or disease sites; an image contrast agent for tracking of movement and accumulation of the particles round the body. Such multifunctional particles could also be tailored in size for delivery to different desired sites, tissues or cells. A major effort towards successful nanoparticle-based therapeutics will be to avoid extensive and non specific immunostimulatory or immunosuppressive reactions to the nanomaterials once administered into the body. Also, these developments bring some degree of risk with it, and to bring a product to the clinic entails identifying all possible hazards, characterizing and quantifying the associated risks, including probabilities and severities, given current scientific knowledge, reducing risks to an acceptable level, balancing any remaining risk against benefit to the patient, and communicating effectively and appropriately on the nature of such remaining or "residual" risks. This implies the future development of new or adapted methods appropriate to assess new medicinal products and devices involving silver nanoparticles. Although many questions still require thorough investigation, the available data suggest that silver nanoparticles can be engineered to become part of the next generation of biocompatible drug delivery platforms.

6. Glossary

- MHC: The major histocompatibility complex is expressed on the surface of cells and displays self and non self antigens to T cells, primarily with the goal of eliminating foreign organisms and other invaders that can result in disease.
- APC: The antigen-presenting cell or accessory cell displays foreign antigen complex with MHC on its surface. T-cells may recognize this complex using their T-cell receptor (TCR).
- TLRs: Toll-like receptors recognize molecules that are broadly shared by pathogens but distinguishable from host molecules and activate immune cell responses.
- ROS: Reactive oxygen species are molecules or ions that are highly reactive due to the incomplete one-electron reduction of oxygen. These reactive oxygen intermediates include oxygen ions, superoxides, peroxides and free radicals.

- Gram +: Gram-positive bacteria retain the violet stain used in Gram's method. This is characteristic of bacteria that have a cell wall composed of a thick layer of peptidologlycan.
- Gram -: Gram-negative bacteria do not retain the violet stain (and take the color of the red counterstain) in Gram's method of staining. This is characteristic of bacteria that have a cell wall composed of a thin layer of peptydoglycan.
- RT-PCR: a high sensitive technique used for the detection and quantification of DNA or messenger RNA (mRNA) in a sample. This technique consists of two parts, the synthesis of cDNA (complementary DNA), a reaction applied when the target sequence is RNA by reverse transcription (RT) and the amplification of a specific cDNA by the polymerase chain reaction (PCR).

7. References

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