Chapter

Nanosilver in Biomedicine: Advantages and Restrictions

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Abstract

Nanosilver (in a range 1–100 nm) binds with thyol-, amino- and carboxy-groups of aminoacid residues of proteins and nucleic acids, thus providing inactivation of pathogenic multidrug-resistant microorganisms. Besides antibacterial, antiviral, antifungal and anti-cancer properties Ag-based nanomaterials possess anti-inflammatory, anti-angiogenesis and antiplatelet features. Drug efficacy depends on their stability, toxicity and host immune response. Citrate coated Ag nanoparticles (NPs) remain stable colloid solutions in deionized water but not in the presence of ions due to replacement of Ag^+ by electrolyte ions, potential formation of insoluble AgCl, subsequent catalyzed oxidative corrosion of Ag and further dissolution of surface layer of Ag_2O. Protein shells protect core of AgNPs from oxidation, dissolution, aggregation and provide specific interactions with ligands. These nanoconjugates can be used for immunoassays and diagnostics but the sensitivity threshold does not exceed 10 pg Cytotoxicity of AgNPs conjugated with proteins is associated with the rate of intracellular Ag^+ release, a 'Trojan horse' effect, and exceeds one of Ag^+ because of endocytosis uptake of NPs but not ions. Relatively toxic nanosilver causes immunosuppression of the majority of cytokines with a few exceptions (IL-1β, G-CSF, MCP-1) whereas $AgNO_3$ additionally activate TNFα and IL8 gene expression.

Keywords: silver ions, nanoclusters, nanoparticles, nanoconjugates with silver core and protein shells, stability, cytotoxicity, immunosupression

1. Introduction

Nanosilver is a generic term that refers to nanoscale Ag materials that have at least one dimension less than 100 nm, and which are commonly in the form of particles called silver nanoparticles (AgNPs). They remain the most used nanostructures in commercialized products. Approximately 320 tons of AgNPs are manufactured each year [1]. There are nearly 500 consumer products that claim to contain nanosilver. At present they are included in nanomedical devices, as tools for medical imaging and biosensing [2] which are used for diagnostics. AgNPs are also employed as antifungal, antibacterial and antiviral drugs [3], for wound dressings and long-term burn care products, anti-bacterial cosmetic lotions for both treatment and supplementary drug and/or nutrient delivery [2]. Besides broad implementation of the nanosilver in health care systems for diagnostic and therapy purposes, medical device coating, medical textiles, contraceptive devices, Ag-containing nanostructures are currently used in cosmetics, clothing, household and food products.

The antimicrobial mechanisms of AgNPs include adhesion to cell surface altering the membrane properties, the formation of free radicals damaging the bacterial membranes and viral envelopes, interactions with DNA, and enzyme deterioration [4]. Besides that oxidative stress induction, heavy metal ion release that occurs in aqueous solutions, producing biologically active Ag^+ [5] and non-oxidative mechanisms were suggested for silver nanostructures [6]. The generation of reactive oxygen species (ROS) inhibits the antioxidant defense system and causes mechanical disruptions of the viral envelopes and cellular membranes. Metal ions are slowly released from metal oxide and are absorbed through the cell membranes or viral envelopes, followed by direct interaction with the functional groups of proteins and nucleic acids, such as mercapto (–SH), amino ($–NH_2$), and carboxyl (–COOH) groups, damaging enzyme activity, changing their structure, affecting the normal physiological processes, and ultimately inhibiting the pathogens of different origin. Currently additional mechanisms of Ag^+ antimicrobial action are becoming evident. Ag^+ ions may react with phosphorus and sulfur groups of surface proteins of the cellular membranes, bacterial cell wall as well as virions after posttranslation modification. Ag^+ binds to negative parts of the membranes including viral envelopes, making a hole. Ag^+ ions damage cytochrome of electron transport chain, impass and destroy RNA and DNA. Ag^+ hinders DNA replication. Ag^+ prevents translation of protein due to damage of ribosomal 30S subunits. Ag^+ ions are sources for the formation ROS that have harmful effect to both eukaryotic and bacterial cells. However, the impact of metal ions on the pH inside membrane coated vesicles is small and has weak antimicrobial activity. Therefore, dissolved metal ions are not determined the main antimicrobial mechanism of AgNPs. Moreover, heavy metal ions can indirectly act as carriers of antimicrobial substances [6]. Thus, disruption of the cellular membranes and viral envelopes, interactions with proteins and nucleic asids [6] are the majors known processes of silver-induced disinfecting activity. These three independent mechanisms take place simultaneously with reversible equilibrium between AgNPs with permanent liberation of Ag^+ ions and reverse deposition of AgNPs from recovered ions and nanoclusters in cells. The numerous mechanisms of action against infectious agents would require multiple simultaneous gene mutations for resistance to develop; therefore, a resistance to silver-containing compounds and nanostructures is hardly possible [6].

Despite the evergrowing presence of Ag-containing products in the market and extensive reports on the antimicrobial activity of AgNPs, insufficient data are currently available about the principal restrictions for the nanosilver to use as diagnostic and therapeutic agents. Inevitably, from the rapid growth in its manufacture and utilization follows an increased environmental and human exposure, whereas the potential acute and chronic toxicity has yet to be fully addressed.

Current research is to analyze stability, cytotoxicity and immunomodulation potential of Ag^+ ions and NPs.

2. Stability of Ag^+ ions, citrate coated AgNPs and their nanoconjugates with proteins

AgNPs in the presence of ions and especially after addition of EDTA are not stable due to oxidation, dissolution and aggregation during a few hours. UV–visible spectroscopy, dynamic light scattering (DLS) and scanning electron microscopy (SEM) revealed that the citrate coated AgNPs remained stable colloid solutions in deionized water at room temperature for decades but not in the presence of ions. Citrate coated AgNPs with the surface Ag_2O layer are not stable in the presence of phosphate buffer solution (PBS) (0.01 M Na_2HPO_4/KH_2PO_4,

0.15 M NaCl/KCl) during 1 hour at room temperature (**Figure 1**) due to replacement of Ag^+ by electrolyte ions, potential formation of insoluble AgCl, subsequent catalyzed oxidative corrosion of Ag and further dissolution of surface layer of Ag_2O [7, 8]. To prevent AgNPs dissolution and aggregation various surfactants and polymers are introduced during or after synthesis [7]. Coating layers may enhance electrostatic and steric repulsion. Adsorption of polymers or nonionic surfactants provides steric hindrances depending upon the thickness of the adsorbed layer [7]. Nanosilver like other NPs immediately after administration into organism becomes wrapped by serum and cellular proteins constituting the protein corona. This protein shells decrease the efficiency of targeting by directing the NPs to the reticuloendothelial system, by masking the active targeting moieties and decreasing their ability to bind to their target receptor, but may re-direct NPs towards endogenous targets. The NPs stability depends on the affinity of coating molecules to the particle surface, repulsion from neighboring molecules, loss of chain entropy upon adsorption, and also nonspecific dipole interactions between the macromolecule, the solvent, and the surface. Protein corona protect AgNPs from dissolution and aggregation (**Figure 1**). The nanoconjugates of the noble metal NPs with proteins remain stable at +4 °C for several months [8]. Surface of nanosilver dynamically adsorbs proteins forming a robust rapidly exchanging "biocorona". A hard corona with long-term stability can be formed with immunoglobulins IgG/IgM and fibrinogens and may alter NPs size, shape, surface charge and agglomeration state, as well as cellular toxicity and internalization, trafficking, opsonization and eventually pattern of biodistribution [8]. Colloids of Ag possess high affinity for binding with serum albumins, their ability to bind with *Staphylococcus aureus* protein A is less efficient, whereas a number of proteins (for example, human immunodeficiency virus (HIV-1) envelope antigen) cannot attach to AgNPs at all. Despite known chemical affinity of sulfur atoms to precious metals direct correlation between cystine disulfide bridge content and binding with AgNPs was not observed perhaps because of strong bonds between two cysteines that stabilize protein conformation.

$AgNO_3$ and its water solutions should be stored in the dark because of possible recovery of silver atoms with formation of nanostructures. Thus, fluorescent metal nanoclusters with sizes less than 2 nm consisting of a few silver atoms can be recovered from Ag^+ in the presence of proteins (albumins, immunoglobulins of different classes and origin and $NaBH_4$ (unpublished data).

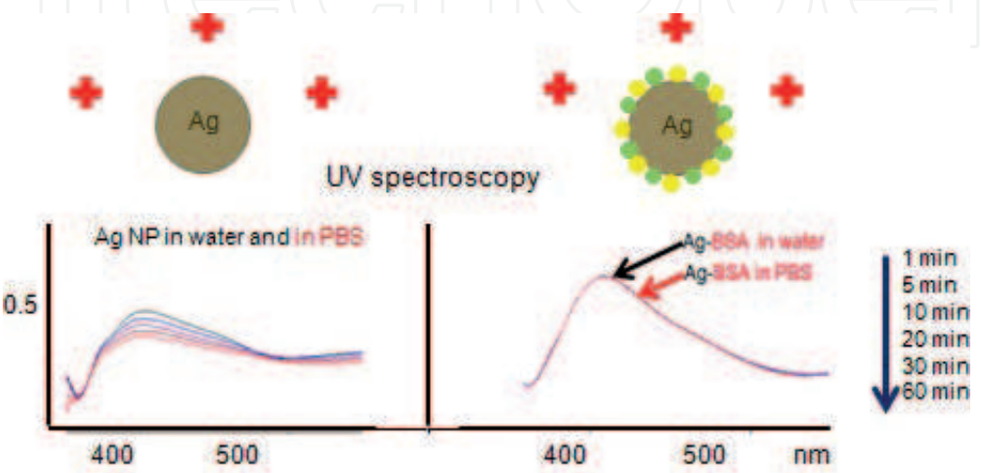

Figure 1.
Stability of citrate-coated and protein-coated AgNPs in deionized water and phosphate buffer solution (PBS) (0.01 M Na_2HPO_3/K_2HPO_4, 0.15 M NaCl/KCl).

3. Nanosilver in immunodiagnostics

Physicochemical features of the nanosilver determine possible implementation in diagnostics. Typical size range of AgNPs 30–80 nm provides high surface to volume ratio. Binding of AgNPs with NH_2- and SH-groups of proteins is weaker compared to AuNPs but protein corona can be formed with the majority of proteins including the main blood proteins. However, leaking Ag^+ cations may damage proteins of envelopes. Extinction, light scattering, surface plasmon resonance (SPR) and SERS of AgNPs exceed those of AuNPs in 10 and 100 times, respectively. Relatively low price is also an advantage of the nanosilver.

The stable nanoconjugates of AgNPs with immunoglobulins of different origin, classes and specificity including both polyclonal and monoclonal antibodies were constructed by: (1) direct binding of AgNPs with purified IgG or IgM [8]; (2) nanoprecipitation of proteins from their solutions in fluoroalcohols [9]; (3) physisorption of proteins on the AgNPs surface treated with poly(allylamine)s; (4) encapsulation of AgNPs into SiO_2 envelope and functionalization with organosilanes. Adsorption of proteins on surfaces of AgNPs is reversible and up to 70% of the attached proteins can be eluted. AgNPs possess high affinity for binding with immunoglobulins but not with any protein. SiO_2 layer on surfaces of metal NPs is suitable for silanization and covalent attachment of any protein. Protein corona prevents AgNPs from oxidation, dissolution and aggregation. The developed methods of fabrication of AgNPs with protein shells permit to attach any protein at different distances from metal core to avoid possible inactivation of proteins, to reduce fluorescence fading and to stabilize the nanoconjugates [8].

To detect binding of immobilized antigens in chip with nanosilver conjugated with IgG the analyzer based on light scattering of dark field laser of total internal reflection with the wave length 532 nm and corresponding software were used. The sensitivity limit of the nanosilver-based immunodiagnostic systems was nearly 10 pg/dot for direct binding of AgNPs with immobilized IgG and 100 pg for 3-layer sandwich immunoassay. For comparison, thresholds of commercially available conventional ELISA and xMAP multiplex immunofluorescent analysis with fluorescent magnetic microspheres were 1 pg/ml. Specificity of Ag nanoconjugates is limited due to their binding with the major blood serum proteins: IgM, IgG, fibrinogen and albumins with increased background level. Protein dots on NH_2- and COOH-modified surfaces of chips are not homogenous causing problems of dot-to-dot reproducibility.

Taken together, immunodiagnostics based on AgNPs covered with IgG shells yields to specificity and sensitivity of the widely used ELISA and xMAP in 10–100 times. Specificity of immunodetection and ratio of signal to background are limited because of binding between AgNPs and blood proteins. Besides the nanoconjugates of AgNPs with protein shells, fluorescent silver nanoclusters containing a few recovered Ag atoms with sizes less than 2 nm can be used in immunofluorescent diagnostics.

4. Cytotoxicity of Ag ions and nanoconjugates of AgNPs with major blood proteins

The common mass-only dose metric model employed in toxicology for traditional substances is not convenient for engineered nanomaterials. Alternative dose metrics include particle number, ion release (kinetics, equilibrium), and the total particle surface area. Nevertheless, polydisperse particle suspensions, the ambiguity in the surface area and concentrations will obscure the analysis. Therefore, Organisation for Economic Cooperation and Development recommended that particle number, surface area, and mass should all be measured when possible to enable calculation

of alternative dose metrics. For AgNPs, both surface area and ion release have been reported as effective alternative dose metrics for nanotoxicological assessment.

Silver in ionic, nanoparticulate, and bulk forms behave very differently. Due to large surface area AgNPs are able for rapid oxidation, dissolution, reactive capacity and binding with biomolecules [10]. When the size of metallic silver is shrunk to nanometre scale, it can enter the cells and cause adverse health effects [10]. AgNPs enter into eukaryotic cells either by endosomal uptake or by diffusion. They can penetrate in living organisms via several routes including inhalation, oral ingestion, intravenous injection, and dermal contact. The American Conference of Governmental Industrial Hygienists has established threshold limit values for metallic silver (0.1 mg/m^3) and soluble compounds of silver (0.01 mg/m^3). Long exposure of humans to the nanosilver from cations to NPs through oral and inhalation routes can lead to argyria, or skin discoloration, and argyrosis, or discoloration of the eyes, as soluble silver incorporates into the tissues with permanent damage of skin microvessels and eyes [11]. Studies *in vivo* with experimental animals have revealed AgNPs accumulation in their liver, spleen, and lung. Similarly, AgNPs-mediated cytotoxicity in mammalian cells depends greatly on the nanoparticle size, shape, surface charge, dosage, oxidation state, and agglomeration condition as well as the cell type. Smaller AgNPs cause more toxicity than larger ones owing to their larger surface area and reactivity [11]. However, currently available data about toxicity of silver nanowires (AgNW) (micron-range long with diameters <100 nm) remain contradictory [11]. For both short (1.5 mm) and long (10 mm) AgNW after inhalation lung inflammation at day 1, disappearing by day 21 has been described, and in bronchoalveolar lavage fluid, long AgNW cause neutrophilic and macrophagic inflammation [12].

Exposure to different forms of the silver leads to distinct outcomes. Whereas elemental silver exposure is not associated with health effects, soluble silver is associated with lowered blood pressure, diarrhoea, respiratory irritation, and fatty degeneration in the liver and kidneys. Furthermore, after different routes of administration including intravenous, intraperitoneal, and intratracheal ways the AgNPs can cross the brain blood barrier *in vivo* and tend to accumulate in liver, spleen, kidney and brain [9].

Respiratory tract, gastrointestinal tract, skin, and female genital tract are the main entry portals of nanosilver into the human body through direct substance exchange with the environment. Additionally, systemic administration is also a potential route of entry, since colloidal silver nanoparticles have been exploited for diagnostic imaging or therapeutic purposes. Inhalation and instillation experiments in rats showed that low concentration, but detectable, ultrafine silver (14.6 ± 1.0 nm) appeared in the lung and was subsequently distributed to the blood and other organs, such as heart, liver, kidney, and even brain. Nanosilver accumulates in blood, liver, lungs, kidneys, stomach, testes, and brain. AgNPs less than 12 nm affect early development of fish embryos, cause chromosomal aberrations and DNA damage.

Animal and human studies indicate that it is difficult to remove silver completely once it has been deposited in the body; however, nanosilver can be excreted through the hair, urine, and feces.

Human liver cells may develop a metabolic-based protection mechanism against AgNPs and Ag^+. The nanosilver penetration through the blood–brain barrier is still debatable. However, even in the absence of Ag in cerebrospinal fluid, Ag-mediated neurotoxic complications such as hypoactivity or reverse increased vivacity, changes in noradrenaline, dopamine and 5-HT concentrations in the brain were observed. Upon oral exposure to $AgNO_3$, the main target organs include liver and spleen, followed by testes, kidney, brain and lungs, and AgNPs are formed *in vivo* from Ag^+ ions and they are probably composed of silver salts. The elimination of

silver from brain and testes is extremely slow [12]. AgNPs may translocate into the central nervous system through damaged blood–brain barrier, nerve afferent signaling and eye-to-brain ways, and even through olfactory receptors of the brain neurons. NPs could stimulate the activation of glial cells to release proinflammatory cytokines and generate reactive oxygen species and nitric oxide production, resulting in the neuroinflammation, including several immune response relevant signaling pathways [13]. While Ag_2S deposits have been seen in the region of cutaneous nerves, there is no indication of toxic risk of silver to the peripheral nervous system [12]. *In vitro* data indicate that silver ions alter mitochondrial function, resulting in the release of apoptogenic signals and subsequent cell death. Moreover, other studies show dose-dependent effects of silver ion on cell replication and other developmental endpoints in mammalian cells [12].

Evergrowing production of AgNPs increases their release into aquatic environments. Once AgNPs get to freshwater, sea or underground water, they oxidize into Ag^+ ions that are toxic to aquatic organisms. Later on, silver cations can bind with abundant anions available in environment with formation of sparingly soluble salts AgCl or Ag_2S. Marine inhabitants (shrimps, prawns, crabs, lobsters and crayfish), are known to be much more vulnerable to the impacts of silver than bacteria [12]. By accumulating in aquatic organisms, AgNPs can enter the human body.

AgNPs have been reported to be toxic to human cell lines [11]. Cellular uptake of AgNPs takes place either via diffusion (translocation), endocytosis or phagocytosis. Upon entering the cytoplasm, AgNPs themselves or Ag^+ ions can generate ROS, leading to DNA damage, protein denaturation, and apoptosis. AgNPs of different sizes and shapes tend to accumulate in the mitochondria, thereby inducing mitochondrial dysfunction, i.e., a reduction in mitochondrial membrane potential (MMP), and promoting ROS creation [11]. AgNPs cytotoxicity in mammalian cells depends on the NPs sizes, shape, surface charge, dosage, oxidation state, agglomeration condition and cell type. They induce a dose-, size- and time-dependent cytotoxicity, particularly for NPs with sizes less 10 nm.

Surface charge of AgNPs stabilized with citrate anions or protein envelopes is a parameter responsible for cellular uptake. In particular, high-level toxicity of positively-charged nanoconjugates versus negatively-charged coatings has been reported. It can be caused by the adhesion of AgNPs onto the negatively charged cell membranes, their consequent entry to the cell, potential release of Ag^+ inside the cell, damage of cellular proteins and nucleic acids and other cytotoxic effects. For instance, the following coatings posses surface charges: (1) positive: polyethylenimine, chitosan, poly-L-lysine and cetyltrimethylammonium bromide; (2) negative: bovine serum albumin (BSA), citrate, odium bis(2-ethylhexyl)-sulfosuccinate; (3) neutral: polyvinylpyrrolidone.

Ag^+ ions alter mitochondrial function, resulting in the release of apoptogenic signals and subsequent cell death. They may destroy DNA-dependent DNA replication, RNA transcription and translation.

Various cellular defense mechanisms, innate immunity of vertebrates and accumulation in certain organs for metabolic-based degradation and subsequent elimination of the nanosilver provide relative protection.

Endocytosis and exocytosis of AgNPs occur simultaneously and depend on physicochemical properties of NPs and protein corona [14]. All nanostructures preferentially accumulate in tumor cells due to the enhanced permeability and retention (EPR) effect. The tumors possess a leaky vasculature and lack lymphatic drainage allowing the drug-loaded NPs to reside at the tumor site for a longer duration compared to the free drug molecules [14].

The formation of protein corona on NPs' surfaces upon the *in vivo* administration is inevitable. Protein shells of all nanostructures after their systemic

administration may explain the lack of the *in vitro-in vivo* correlation and the preclinical to clinical extrapolation. Protein shells provide stability of the nanoconjugates, decrease their cytotoxicity and determine the interaction of NPs with the target and non-target cells. The chemical composition of protein corona may serve as a fingerprint for NPs of certain type since different NPs tend to recruit cellular and serum proteins to variable extents. Vitronectin mediates accumulation in integrin receptor-expressing melanoma cells both *in vitro* and *in vivo*, while complement 3 protein (C3) and, as an opsonin and dysopsonin, regulate the balance between the the reticuloendothelial system uptake and blood circulation [14].

The cytotoxic effect and oxidative stress of silver ions (Ag^+) on mouse lung macrophages cells resulted in necrotic rather than apoptotic cell death by reducing functional sulfhydryl groups in the cells [12].

Other defense mechanism performed by the most abundant leukocytes - polymorphonuclear neutrophils (PMN) is based on neutrophil extracellular traps (NETs) [15]. NETs are extracellular fibers consisting of DNA with histones (H3), myeloperoxidase and neutrophil elastase. NETs form a barrier that hinders the transmission of pathogens and due to high local concentrations of antimicrobial proteins degrade virulence factors and kill bacteria. However, high concentrations of active proteins may cause host immune damage, contribute to platelet aggregation and cause thrombosis [15, 16]. The nanosilver activates polymorphonuclear neutrophils to release NETs did not alter the extracellular lactate dehydrogenase level [16].

Noteworthy, that the nanosilver toxicity exceeds one of Ag^+. For instance, cytotoxic concentration of $AgNO_3$ for 50% of human larynx carcinoma HEp-2 cells (CC_{50}) was 50 μg/ml, whereas for the nanoconjugates of AgNPs with surface proteins CC_{50} = 0.1 o.u. = 1.4 μg/ml (calculations are based on the calibration curve of atomic absorption spectroscopy (AAS) data). One of possible explanations is AgNPs limited cellular uptake. At present there is not evidence of the efficient uptake or intracellular localization of the citrate coated AgNPs conjugated with fluorescent proteins. Intracellular Ag^+ release appears to be responsible for the toxicity since the cultural media after treatment of cells with the nanosilver do not cause any cytotoxicity. Thus, AgNPs were toxic for mammalian cells and the cytotoxicity is associated with the rate of intracellular Ag release, a 'Trojan horse' effect [1, 9].

5. Inhibition of innate immunity with the nanosilver

Cytotoxicity of the nanosilver evidently determines host defence such as apoptosis, necrosis [2] and NETs formation [16]. Moreover, AgNPs, but not Ag^+ ions, decrease the viability and the cytotoxic potential of natural killer (NK) cells secreting cytokines and killing damaged cells [12].

Nanosilver possess anti-inflammatory properties in both animal models and in clinic. Thus, AgNPs inhibit the expression of proinflammatory cytokines transforming growth factor (TGF) -β and tumor necrosis factor(TNF) -α. Nanosilver administration attenuates nasal symptoms of allergic rhinitis in mice and inhibites immunoglobulin IgE, IL-4, IL-10. In clinical study, wound dressing containing AgNPs promoted the healing of chronic leg ulcers due to antibacterial effect in the wound and by decreasing inflammatory response. Ability of nanosilver to reduce cytokine release and production of matrix metalloproteinases, decrease lymphocyte and mast cell infiltration, and induce apoptosis in inflammatory cells may explain their anti-inflammatory mechanisms [17].

Innate immunity induction with $AgNO_3$ and AgNPs conjugated with the major blood proteins – albumin, fibrinogen, immunoglobulines was assayed by xMAP with fluorescent magnetic microspheres.

Inflammation biomarkers	Th1				Th2								Th17	Others			
	IFNγ	TNFα	IL-1β	IL-12(p70)	IL-2	IL-4	IL-5	IL-6	IL-7	IL-8	IL-10	IL-13	IL-17A	G-CSF	GM-CSF	MCP-1 (MCAP)	MIP-1β
$AgNO_3$	1.03	1.15 ↑	1.38 ↑	0.70	0.74	0.84	0.88	0.85	0.70	3.71 ↑	0.78	0.81	0.97	1.12 ↑	0.92	1.19 ↑	1.17 ↑
AgNP-BSA	0.76	0.72	0.70	0.70	0.82	0.74	0.70	0.75	0.50	0.64	0.78	0.69	0.76	0.78	0.79	0.85	0.76
AgNP-Fb	0.72	0.65	1.00	0.72	0.78	0.75	0.82	0.75	0.55	1.00	0.77	0.83	0.84	1.00	0.83	1.15 ↑	0.97
AgNP-hIgG	0.68	0.51	2.11 ↑	0.54	0.48	0.43	0.60	0.41	0.44	0.34	0.54	0.50	0.97	1.20 ↑	0.83	0.76	0.66

Table 1.
Multiplex immunofluorescent xMAP data for 17 inflammation biomarkers in HEp-2 cells in 2 days posttreatment with 5 μg/ml $AgNO_3$ and 0.02 o.u. of nanoconjugates of AgNP-with BSA, fibrinogen (Fb) or hIgG. Normalization was carried out as a ratio of mean fluorescence intensity (MFI) of fluorescent magnetic beads after treatment with Ag ions or nanoconjugates to MFI of control intact HEp-2 cells.

The maximal production of all 17 studied biomarkers including T-helper (Th)1 cytokines: interferon (IFN) γ, tumour necrosis factor (TNF) α, interleukin (IL-1β), IL-12 (p70); Th2 cytokines: IL-2, 4, 5, 6, 7, 8, 10, 13; Th17 – IL-17A and other inflammation biomarkers: granulocyte colony-stimulating factor (G-CSF), granulocyte macrophage colony stimulating factor (GM-CSF), Monocyte Chemotactic Protein 1 (Monocyte Chemotactic And Activating Factor) (MCP-1 (MCAF)), Macrophage Inflammatory Protein 1β (MIP-1β) was registered during first two days posttreatment. In HEp-2 human cell line in 2 days posttreatment with 5 μg/ml AgNO3 (for comparison, cytotoxic concentration for 50% cells (CC_{50}) of $AgNO_3$ for HEp-2 cells is 50 μg/ml) the significant up regulation was registrated for IL-1β (slight increase in 1.38 times up to 0.08 pg/ml), IL-8 (significant growth in 3.7-times up to 0.94 pg/ml) whereas steady production of TNF α (growth in 1.15 times), G-CSF (up in 1.12) MCP-1 (MCAP) (increase in 1.17 times up to 0.39 pg/ml) was observed; down regulation – for regulatory IL-10 (below the IL-10 production in control intact cells with less than 0.05 pg/ml and therefore undetectable in ELISA with detection limit 1 pg/ml) (**Table 1**). IL production is known to depend on the origin of human cells. Thus, IL-1β is mainly produced by macrophages and monocytes but HEp-2 cell line is derived from larynx carcinoma cells and HT-29 – from human colorectal adenocarcinoma cells. IL-1β is responsible for initiation and regulation of inflammation, stimulation of acute phase cytokines such as IL-2, −3, −6, TNF-α as well as for temperature growth and fever. Therefore, inflammation is hardly possible with low level of IL-1β and stimulated cytokines. The only exception was IL-1β-induced up-regulation of IL-8 (**Table 1**) which is associated with acute and chronic inflammation. Silver ions at concentration lower CC_{50} were not toxic for the human cell line and could not penetrate through membranes in cells. Therefore, the observed immunomodulation with $AgNO_3$ was so modest (if any).

Immunomodulation with nanoconjugates of AgNPs covered with the major blood proteins: albumins, fibrinogen, or immunoglobulins differed (**Table 1**). Significant increase in 2.11-times of IL-1β production (up to 0.12 pg/ml) and G-CSF (in 1.20-times) was detected in 2 days after treatment of HEp-2 cells with AgNPs-hIgG simultaneously and similar to its growth after $AgNO_3$ addition. However, the growth did not stimulate production of other cytokines (**Table 1**). Growth of MCP-1 (MCAP) secretion (up to 0.37 pg/ml) was caused by AgNPs-fibrinogen. Inhibition of IL-8 and regulatory IL-10 production in the presence of all nanoconjugates with Ag core and protein shells resulted in slight changes (if any) of 17 biomarkers. Noteworthy that AgNPs-BSA caused decreased production of all inflammation biomarkers studied (**Table 1**). However, the lowest concentrations below 50% of the corresponding values in control cells were found after treatment with AgNPs-hIgG perhaps due to reverse regulation of innate immine response with antibodies.

6. Conclusion

Limited stability of nanosilver without stabilizing envelopes in biologically relevant media, cytotoxicity for both eukaryotic and bacterial cells, negligible cellular uptake restrict their further implementation for combined antiviral and antibacterial therapy. However, spontaneous binding with the major blood proteins and anti-inflammatory properties with inhibition of cytokine production at the early stages after treatment may be helpful in prevention of cytokine storm caused by RNA-containing viruses.

Conflict of interest

The authors declare no conflict of interest.

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