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PARADOXICAL AFFINITY OF SELF SERUM CAPPED ANIONIC SILVER NANOPARTICLES TOWARDS HIGH NEGATIVE CHARGED MICROBES AND CANCER CELLS

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ABSTRACT

Following intravenous use of Colloidal silver nanoparticles (AgNPs) intended for target specific delivery of a bulk toxic atoms, satisfactory outcome essentially demands haemo-compatibility and wide margin of safety to the host. Well buffered, matched serum can be source of best tolerable capping and reducing agents for such therapeutic purposes. Chance of materialistic state alterations in-vivo by secondary "corona protein cap" formation can be eliminated by using homologous serum. This generates primary corona protein capped novel nanoparticles with wide cluster range, smaller, electrically neutral, or weak negative charge. In prokaryotes, initially small size range AgNPs may overcome weak repelling force and disintegrate cell membrane at contact point by mobilizing positive ions into outer diffuse layer of AgNPs, due to increasing intensity of van der Waals force. Subsequently larger AgNPs can enter through wider membrane-gaps created from inside by super-oxide mediated membrane lipid-peroxidation. Selectivity may be further enhanced by adding target specific antibodies. Anti-biofilm and nonspecific synergism properties of personalized AgNPs, can also be advantageously used for therapeutic purposes. Same nanoproducts may be selectively internalized by receptor-ligand based endocytosis into strong negative surface charge bearing blood parasites, yeasts, and cancer cells, sparing host cells with identical molecular sign on surface.

KEYWORDS: Silver nanoparticles, Charge-sign, Molecular-sign, Corona protein cap, Margin of safety, Personalized nanomedicine.

INTRODUCTION

Viewpoint on topic

Recent high alarming trend of multi-drug resistant microbial infections has pushed scientists to the door-step of "post-antibiotic era" and demands a breakthrough by introducing newer strong wide range but reasonably resistance-proof agent.[1,2,3] Suitably designed blood-compatible heavy metal colloidal nanoparticles (NPs) may be such gamechanger agents for their unique physicochemical affinity based multimode toxic actions on accessible cells with different marker identities. Accessibility into bacterial cells can occur following charge sign dependent membrane attachment of smaller NPs with localised disintegration.[4,5] Internalization of little larger NPs occur in eukaryotes following receptor-ligand based endocytosis[6], where both charge sign and molecular sign play important role. Theoretically colloidal NPs with positive zetapotential > 10 mV, like polyethylene-imine stabilized AgNPs can firmly attach on high negative (ZP > –30 mV) surface-charge bearing bacterial membrane[7], but those cannot be used for common therapeutic purposes for risk of mis-targeting many highly replicating host-cells along with cancer cells. Paradoxically, many workers[8,9] have observed strong antibacterial action of weak anionic AgNPs (ZP < –30 mV), even by electron microscopic imaging. Such phenomenon [Fig: 1] can be explained by resultant positive ion mobilization at approaching narrow contact zone of NPs into outer diffuse layer, by influence of van der Waals attractive forces, abiding by DLVO (Derjaguin-Landau-Verwey-Overbeek) theory.[10,11] Siritongsuk et-al have demonstrated a rapid killing phase of bacteria within 5±30 min. due to rapid neutralization of surface charge from low negative range to low positive range, followed by late killing phase of 1±4 hr after the AgNP treatment due to excessive production of Reactive Oxygen Species (ROS) in cytosol.[12] ROS can induce toxicity by inter acting with different bio-molecules, including lipid peroxidation of membrane, leading to cell death by osmotic disbalance, leakage of cytoplasmic contents and further influx of larger size AgNPs.

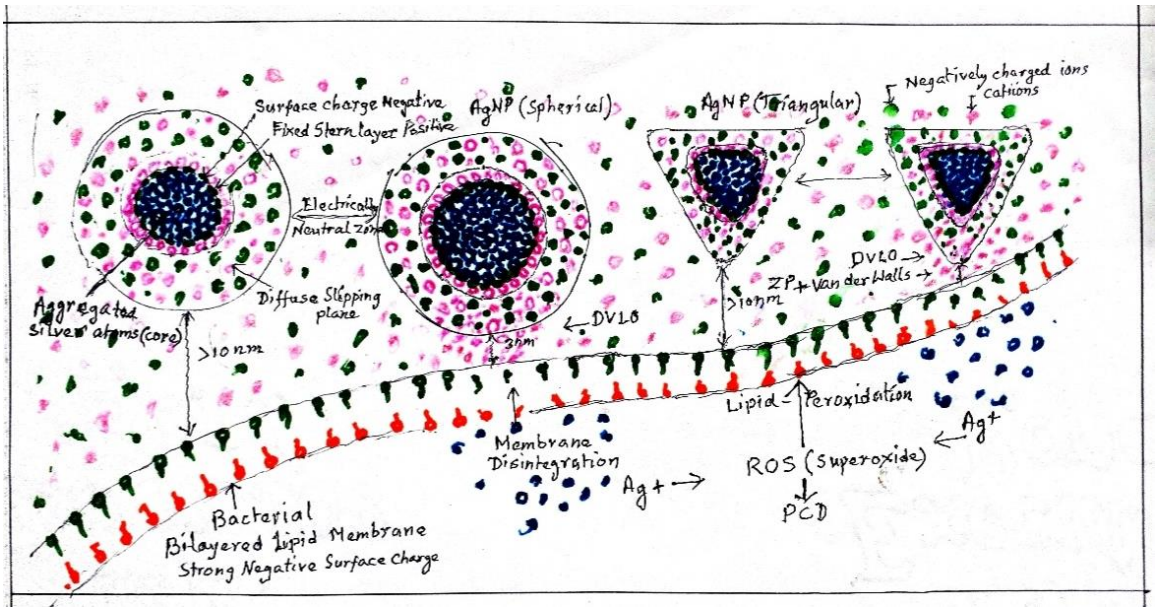

Fig. 1: Internalization of anionic AgNPs by disintegration of negatively charged bacterial cell membrane, due to DVLO effect and subsequent ROS mediated membrane lipid-peroxidation.

As these nanoparticles serve as charge sign and molecular-sign guided prompt target delivery agents for payloads containing huge number of cytotoxic heavy metal atoms, may be called as "Nano Bio-Ballistic Missile." Infused nanoproducts in a stable dispersed colloidal state flow at a tremendous force of about three feet per second with other several hundred times larger blood cells. When those collide with invader microbes or neoplastic cells, can attach to some non-self-surface molecules with high affinity van der Waals electro-magnetic attractive force on interface of each other. Here higher surface: volume ratio of smaller size NPs can serve more efficient target delivery agents[13] for greater availability of contact areas to deliver same volume pay-load. One 50 nm core-size spherical AgNP carries 125 times pay-load present in 10 nm size similar nanoparticle (as ratio of volume ∞ cubic ratio of radius), while 5 times more surface area (as ratio of surface area ∞ square ratio of radius) is available for trafficking by such smaller 125 particles than that of larger one.

However, massive application prospects of so called green-synthesised nanoproducts or haemo-incompatible heavy-metal-based colloidal nanoparticles are meant for use as antiseptics, disinfectants, hand-wash, or pesticides, but are unsuitable for intra-venous use either due to their intolerable capping agents or tendency of bio efficacy alterations in blood-stream after gradual acquiring corona protein caps[14,15,16] with higher affinity plasma protein components. Only a small fraction will be used-up for target-killing in some non-medicinal

uses, while spillage amount may be dangerous for existence of resident microbes in soil and water.[17,18]

In all respect haemo-compatible silver nanoparticles[19] are mostly preferred, those require low temperature chemical reduction to prepare from mono-valent silver by use of homologous blood components, avoiding thermal alteration of capping proteins. Surface zeta-potential (ZP) at near neutral pH of serum attains a neutral range – 8. 9 mV (neutral ZP range between – 10 mV to + 10 mV) and ensures maximum non-interference to healthy host cells as well as colloidal stability in blood. A high negatively charged (ZP > –30 mV) microbes of intractable blood-stream infections or cancer cells with identifiable molecular signature on surface, still can attach with such small, novel AgNPs by resultant electrostatic attractive force abiding by DLVO theory. Even as small as < 15 nm novel AgNPs from cluster size range can diffuse through deranged porin channels in bacteria, while larger particles may follow receptor-ligand based endocytosis into yeast, blood-parasites, or neoplastic cells.

In therapeutic sector of nanomedicine, a specific drug tagged on functionalized carbon nanoproducts have initially gained importance, though those have limited range of action and susceptibility according to the original drug.[20] Heavy-metal based colloidal nanoparticles are relatively omnipotent cytocidal, subject to accessibility into any kind of cells, based on charged-sign and molecular sign dependent affinity. Usually, such NPs are prepared by chemical reduction of ionized metallic salt in solution. The method requires input of high energy to form an unstable compact cluster of heavy-metal atoms with conserved high entropy level. To prevent larger aggregation, various surfactant capping molecules[21] are added, those impart stabilization to the core by strong adsorption. Thus, a colloidal suspension containing nanoparticles ranging one to hundred nm size can be obtained. Each particle consists of a core of negatively charged metal atoms, surrounded by an electric double layer comprising a fixed, positively charged stern layer and diffused surface layer containing positive and negatively charged electrolytes from medium with charged proteins or other surfactant macromolecules. Thus, mutual repelling force separates NPs floating in electrically neutral interface region and prevents agglomeration.

After de-capping, the stability of nanoparticle and ability to release metal ions in cytosol depends upon local pH, nature and concentration of these capping agents.[22] Shape of NPs and core size is determined by electron microscopy, while cluster sizes are obtained from DLS measurement. The potential difference between stationary layer of fluid attached to the

dispersed particle and the dispersion medium is expressed as zeta potential. The colloidal state of NPs is maintained for a long time without agglomeration by mutual negative repulsive force. It only indicates nature of surface charge while actual surface charge density is calculated as the amount of statical-electric charge per unit area of space in one, two or three dimensions with all intermolecular forces. Charge dependent affinity of NPs follows competitive consumption principle on target, so to avoid disproportionately higher lethal dose consumption by small group of first encountered target cells, slow addition with stirring is recommended for obtaining uniform cytotoxic effect.

Present development

For a long-time scientist have identified some unique features of NPs which labels those as "all-in-one solution" to combat two major issues of heath sector, like management of multi-drug resistant infections and cancers. No such product is yet approved for systemic use mainly for safety issues of host and environment. In most of earlier literature many confusions persist about anti-microbial role and pharmacological mechanisms of AgNPs, which in the light of newer observations becoming clearer and scientists are optimistic to find suitable solution.

By detail studies on corona-protein cap, true-identity of NPs during medicinal uses has revealed with understanding reasons for physical, pharmacological, and toxicological possibilities. Now we have information that concentration and nature of protein absorbed on bare NPs depends upon hydrophobicity of the NPs. In blood, different charge affinity proteins are present and start depositing on differently capped NPs immediately after exposure to plasma, leading to corona formation with surface charge alteration and decreased toxicity.[16] Positively charged AgNPs has greater affinity for albumin absorption while negatively charged one shows higher affinity for IgG, though albumin appears as major component of corona proteins for presence of much higher concentration in serum.

This instigates us to develop primary corona protein capped novel AgNPs.[19] Initially, pooled human serum has been used for toxicity studies in mice model and cell lines, keeping a control PVA-capped AgNPs. Greater margin of safety with potent antimicrobial actions have been reported and results are when interpolated with infected human being, about10 mL self-serum derived AgNPs ensures safety level and much higher achievable plasma concentration, sufficient for killing most of MDR strain microbes. This novel AgNP is close to primary corona-proteins capped NPs except fibrinogen and coagulation factors. This can be further

improved by dropping dextrose as reducing agent so that strength of protein concentration and IgG level can be doubled. Serum contains sufficient reducing substances (Glucose, Ascorbic acid 50-60µM, Glutathione 4-5µM, Uric Acid 200-400µM, Lipoic Acid 0.1-0.7µM, Antioxidants like Amino acids, Vit-E, Microelements & intermediate products of metabolism) and cocktail of capping proteins (albumin, globulin, structural proteins, enzymes etc.), that generates nearly 17 nm size AgNPs with – 8.8 mV zeta potential in dextrose added preparation and smaller size range from 5 to 8 nm has been demonstrated by omitting dextrose [Fig-2]. ZP of such serum capped AgNPs is almost equal to surface charge of human erythrocytes[23], so there is least chance of nano-induced haemolysis in circulation and complications thereof.

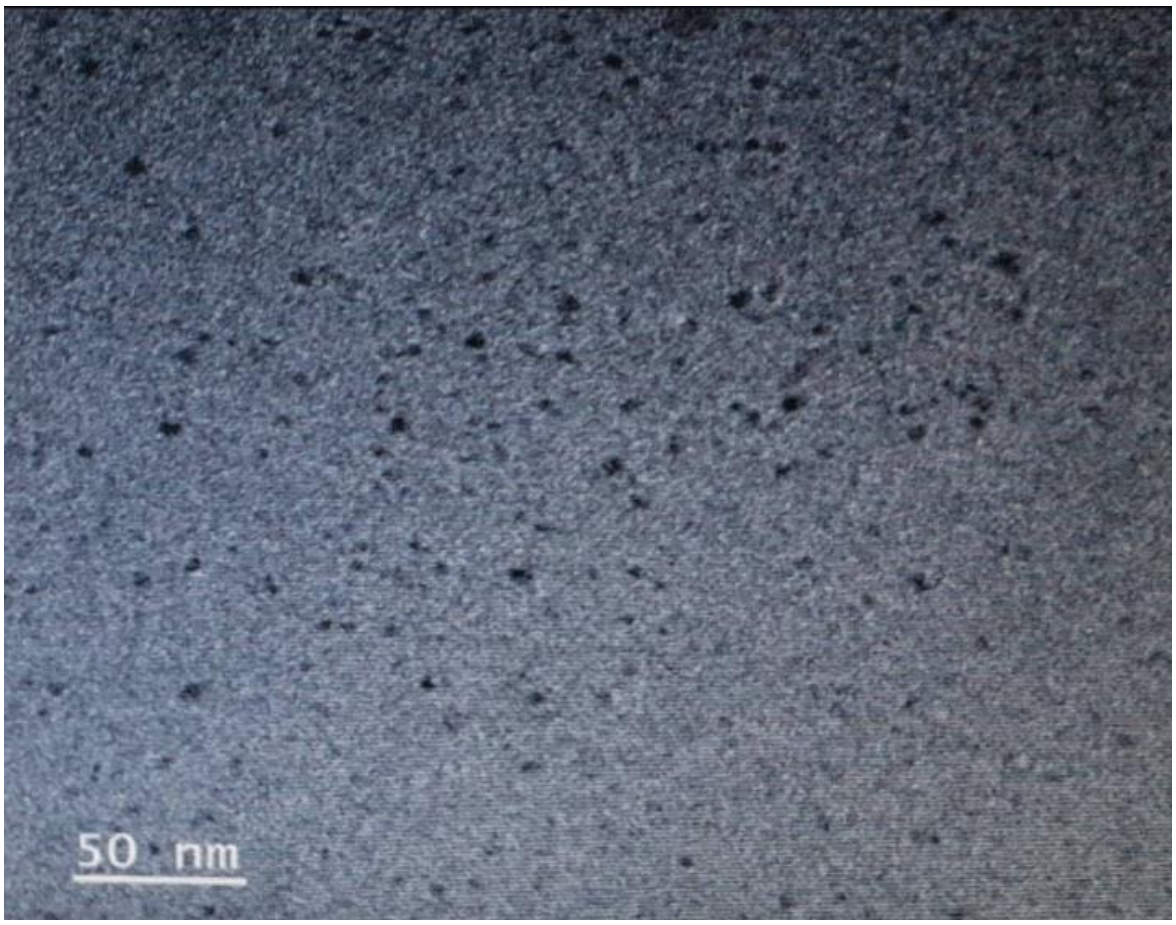

Fig. 2: Variable size and shape of serum capped silver nanoparticles in TEM image.

Molecular signatures of such homologous-serum capped AgNPs need to be thoroughly studied for developing more target selective NPs. Of corona-protein components, specific Immunoglobulin G has selective binding affinity to specific antigen bearing microbes. According to Vroman effect, proteins with higher concentration will be attached first on

surface of nanoparticles within minutes, then gradually will be replaced by higher affinity proteins within an hour. In this respect immunoglobulin being second highest protein in serum after albumin, may attach adequately for their high affinity also. Carboxy terminals of Fc domain are likely attached on nano surface for high affinity, leaving amino-terminals two Fab regions free for antigen binding. This has been verified by our preliminary study with primary corona-protein capped nanoproducts, using Widal test reactive serum and compared with control non-reactive serum. Killing of typhoid isolate *Salmonella sp*. shows significant higher bacterial killing by the former than that of control. Similar tests can be performed after incorporating monoclonal or polyclonal antibodies with capping serum. Antimicrobial polypeptides are also found to enhance specific microbicidal actions when conjugated with AgNPs.[24]

Plasmid mediated heavy-metal resistance is known for a long time. Now it is claimed that bacteria can develop adoptive resistance against metal nanoparticles by various ways for excessive spillage in environment.[25] By using minimum rational dose of personalised novel NPs with or without synergistic use of antibiotics, such probability is practically nil. Any excess amount can be safely disposed-off in hypochlorite solution.[26] Apparently resistant conventional antimicrobial drugs when are tested with 1/4th effective concentration of carboxy-methyl cellulose capped AgNPs, universal synergism has been demonstrated by VITEK automated susceptibility testing device against wide range multidrug resistant bacteria and yeast.[27] This can be explained by higher permeation of these drugs through nano-induced damaged cell membrane. So, personalised nanomedicine should be used by slow infusion as bullous one or two dosages at long intervals with use of conventional anti-replicant antibiotics in between, to avoid pharmacological incompatibility.

Though structurally viruses differ from cells, AgNPs play antiviral role either by altering integrity of receptor binding site or blocking intra-cellular replication following entry with virus after attachment with lipid envelop.[28,29] Small size novel NPs may prove effective if possess charge affinity and / or molecular affinity with any incorporated viral antibody on protein caps. As structural alteration due to corona-protein deposition is not applicable for such NPs, in-vitro anti-viral effects will almost simulate with their in-vivo efficacy.

For common intracellular mechanisms of toxicity, killing of cancer cells may be enhanced by exposure of AgNPs, but charge-sign may play crucial role for safe target selective use. Surface charge of cancer cells is strongly negative while originator cells maintain neutral or

slightly positive charge. Due to high rate of glycolysis, cancer cells secrete about 30 times more lactic acid as metabolic end-product while normal cell mainly produce carbon dioxide and water. As a result, high acidic environment is created in both intracellular and extracellular compartment of tumor mass producing high negative surface charge in all 22 tested cancer cell lines.[30] So, poly-ethylene-imine stabilized AgNP with ZP +55 mV is strongly cytotoxic for cancer cells, but contraindicated for severe toxicity to vital organs. Small size AgNP with weakly anionic or neutral range ZP may be useful. Antimicrobial like cytotoxic pathway has been described on colorectal cell line by 2–10 nm biosynthesized AgNPs.[31] The impact of solvent and pH in synthesizing AgNPs has been tested and documented that in acidic medium (pH < 5) charge of NPs decreases with higher release of ionic silver. In a human pilot study, it has been demonstrated that interaction of tumor charge shifting towards normal by applying non-ionizing radiation with positive electrostatic charge treatment can be a great beneficial complimentary therapy of cancer.[32] So, it is expected that, ZP of 5–10 nm size personalized novel AgNPs may instantly change ZP toward weak positive range in very high acidic extracellular environment of tumor mass and may be internalized by endocytosis to exert cytotoxic effects on cancer cells. Further studies in this regard may add NPs as a nonspecific anticancer drug that can lower dose of synergistically used chemo therapy or radiation. In future, antibody against cancer bio-markers can be tried to incorporate with novel NPs, to enhance target selectivity.

CONCLUSIONS

By developing a safe, selective, omnipotent antimicrobial and anticancer drug for systemic use can minimize diagnostic delay, therapeutic cost, and resistance development possibilities of synergistically used conventional drugs. Thus, modern medicine may find ray of hope to combat two types of important challenging diseases, in the form of nanomedicine, pharmacology point of view which are a new class of drug for their unique materialistic state. All stages of trials are to be carried out using self or homologous serum based nanoproduct as personalized drug. After obtaining approval, rational use policy with possible synergistic drugs is to be formulated according to clinical condition. As DNA level damage on environmental microbes are found to persist in their several progenies, one or two bolus doses may be formulated with use of conventional drugs in between. Side effects, pharmacological incompatibilities, PK-PD like issues are to be critically tested. Improvisation of novel NPs by selective alteration of capping components will not stop.

Declarations

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