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A REVIEW ON NEW INSIGHT OF BIOGENIC PRODUCTION OF Ag NANOPARTICLES (Ag NPs) FOR GEL AND THIN FILM PREPARATION AND THEIR ANTIMICROBIAL APPLICATION: AN ECOLOGICAL APPROACH

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Abstract

In recent years pharmaceutical companies and many research, papers are searching for alternative and efficient antimicrobial agents due to resistance of pathogenic organisms to many antibiotic present. Silver nanoparticles (Ag NPs) play an important alternative antibacterial agent as it has potent antibacterial activity towards microorganisms and shows significantly higher synergistic effect with many antibiotics. The distinctive problem of Ag NPs is the stability and shape which can be resolve by different capping agents. This work will deal the various synthesis parts of Ag NPs, suspensions stability and their applications as antibacterial activity, Ag NPs will synthesis by green synthesis root which will be expected stabilize by plant extract moieties, Ag NPs will synthesis through the microorganism roots using *E.coli*, *Bacillus subtilis, Staphylococcus aureus* and *Pseudomonas aeruginosa*. Various parameters will use to synthesize the Ag NPs like extracellular and intracellular extract based on microorganism, Ag NPs stabilize by PVP at various silver salt concentrations. Anticipatively, different salt concentrations lead different shape and size of Ag NPs which may lead to work as photo-thermal effects. The bacteriocidal activities of Ag NPs appear to be through release of silver ions. These ions induce and impact on the cells as de-energization. Which efflux of intercellular content and disrupt DNA replication followed (individually or in combination) by increased membrane permeability and loss of the proton motive force. Currently the major problem is bio-film formations by microorganism which lead critical adverse effects on the environment as well as human. We discuss the antimicrobial efficacy of Ag NPs against selective pathogens and bio-film. Ag NPs embedded polymer based thin film using different shape and sized Ag NPs. From literature surveys, it has been anticipated to apply these Ag NPs embedded polymer film as antibacterial, antifungal surfaces to inhibit bio-film formations. This new kind of material may also be useful to create microorganism free surface for laboratories and water purification. We review Ag NPs synthesize by various roots and embedded in polymer surface films that characterize by SEM, TEM, AFM, XRD, UV, TGA and FTIR techniques. Further, microorganism and Ag NPs interaction mechanisms will explore and develop on the basis of SEM and TEM data.

Keywords: Silver nanoparticles, Microorganism, SEM, TEM, AFM, XRD, UV, TGA.

1. Introduction

The topic of nanoparticles has received particular importance in a wide range of fields. The term “*nano*” comes from the Greek word “*nanos*” meaning dwarf and denotes a measurement of the scale of one-billionth (10^9) of a meter in size [1]. A strand of DNA is 2.5 nm in diameter [2], a typical virus is around 100 nm wide [3] and a typical bacterium is around 1-3 μm wide [4]. As classical antimicrobial agents have become increasingly less important, and a large proportion that remains effective are highly toxic, making them useless for using in the food, medicine, and textile industries [5]. Certain metals have disinfectant properties of such as silver and their salts are documented in traditional medicines [4, 6]. From centuries silver in ionic form is known to cure venereal diseases, bone and perianal abscesses, eye diseases and burns. And also Ag^+ was active against various bacterial species e.g. *E. coli*, *S. aureus*, *Klebsiella sp.* and *Pseudomonas sp.* [7] the most functionalizing and commercializing nanomaterial are silver nanoparticles due to their unique physicochemical properties (high surface to volume ratio) like electrical [8], optical [9] and particularly antimicrobial properties [10]. The nanoparticles have antibacterial activity and a synergistic effect with erythromycin, methicillin, and ciprofloxacin [11]. It was also found that Silver nanoparticles possess antifungal [12] anti-inflammatory [13] antiviral [14] anti-angiogenesis [15] and antiplatelet activities [16]. Recently an important area of scientific research devoted to synthesis of nanoparticles especially searching for an eco-friendly and green materials for current science. Silver nanoparticles inhibit cell division resulting in damage to cell envelope and cellular contents of the bacteria [17]. Apart from its antimicrobial nature, silver has long been documented as an efficient antioxidant agent as it shows slow toxicity in living organisms and has various in vitro and in vivo applications [18,19]. Various methods like irradiative, chemical, photochemical, physical, electrochemical, and biosynthetic techniques have been approached for the synthesis of metal nanoparticles [20–27]. Even though most of the methods have produced pure and well defined nanoparticle. Among these synthetic methods, the biological synthetic approach has been proven one of the best environments friendly and cost-effective. In biosynthesis of metal nanoparticles large number of biomolecules act as reducing and protecting agents. Biosynthesis of stable nanoparticles has received considerable attention due to the exceptional physicochemical features of nanoparticles, inclusive of magnetic, antibacterial, optical, electronic properties and catalytic activity [28-30]. The use of biological materials in the synthesis of nanoparticles has gained momentum in recent years due to their distinct biomedical, catalytic, photovoltaic and energy applications [31-34]. The surface enhanced resonance properties of silver nanoparticles are being utilized for various biomedical applications as a sole and in combination with other smart materials [35]. Different metal nanomaterials synthesized by different techniques like plant extracts, fungi, bacteria and hard template [36-39]. Among them silver nanomaterials have an important role in chemical, biological and medical sciences owing to its attractive physiochemical properties. The study of plant materials as potential nanofactories has gained much interest in the biosynthesis of nanomaterials. Polymer based stabilized nanoparticles are also carried modified surface properties. Polymer core at nanoparticles provide the particles suspension stability and particular shape and size. Variation in precursor concentration in the system is resulted in various shape and size nanoparticles those are distinctive for biological moieties interaction [40-43]. Polymer based stabilized Ag NPs may drawn in thin film which will have a huge range of application like antibacterial agents [44] implantable biomaterial [45] water treatment [46] molecular imaging, [47] diagnosis and treatment of cardiovascular diseases [48] wound healing, [49] drug delivery [50] and clothing [51]. Earlier studies have suggested that nanosilver induce anti-inflammatory responses by this it can be use as anti-inflammatory agents to inhibition the gamma interferon and alpha tumor necrosis factor which are involved in inflammation [52]. So it can be anti-inflammatory agents, also the plasmonic properties of nanosilver can effectively biosense that makes it active biosensors for a large number of proteins that normal biosensors difficult to detect it. This unique advantage can be utilized for detecting various abnormalities and diseases in the human body including cancer [53-55]. Due to potent application as antimicrobial agent nanosilver used for development of novelchitin/nanosilver composite scaffolds for wound dressing [56], nanosilver are currently being used in many household water purification systems, in many products such as cloths, washers, water purification systems, tooth paste, filters, fabrics, deodorants, shampoo, paints, kitchen utensils, and toys to impart antimicrobial properties [57] (Fig.1).

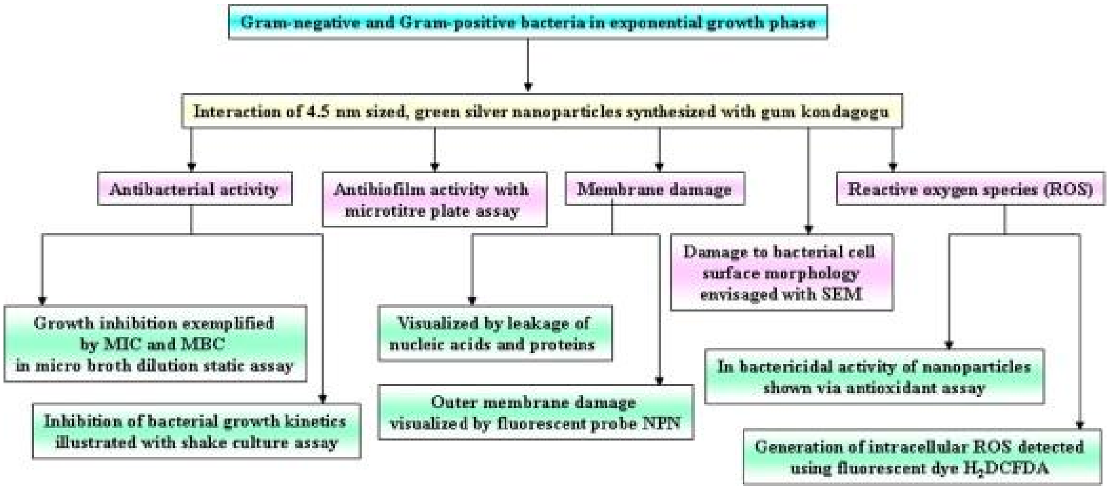

Fig (1) Proposed mode of antibacterial action of silver nanoparticles demonstrated with various susceptibility assays

2. Synthesis of metallic nanoparticles

Nanoparticles are considered to be substances that are less than 100 nm in size in more than one dimension exist in many forms and shapes, fused, aggregated, or agglomerated forms. Spherical, tubular, or irregularly shaped created [58]. Biogenic metallic nanoparticle synthesis can be split into two categories. The first is bioreduction, by reduction of metal ions chemically coupled with the oxidation of an enzyme into more stable forms biologically. Many organisms have the ability to utilize dissimilarly metal reduction [59]. The second category is biosorption that does not require the input of energy it occur by involves the binding of metal ions from an aqueous or soil sample onto the organism itself, such as on the cell wall. Certain bacteria, fungi and plants able to form stable complexes in the form of nanoparticles as they express peptides or have a modified cell wall which binds to metal ions. Due to their potential application in many fields metallic nanoparticles are becoming increasingly important. However, it is crucial for the development of an environment friendly and inexpensive way of synthesizing nanoparticles. There are numerous organisms have the potential to be exploited and modified to optimise them to synthesize nanoparticles [60]. Wiley et al. [61] were able to produce silver nanoparticles (AgNPs) of cube and tetrahedron shape. They achieved this result by heating $AgNO_3$ and Ethylene glycol to 148°C. The resulting nanoparticles were single crystals and had a size range of 20-80 nm in diameter, the reaction time was only 10 minutes [61]. Another method using polyamide fabrics developed by Montazer et al. [62] successfully formed AgNPs. The NPs were observed under SEM and were found to be between 20 and 150 nm across with an average size of 90 nm. Mafuné et al. [63] used laser ablation that creates nanoparticles by heating up bulk material using a laser beam. Research has focused mainly on prokaryotes as a means of producing metallic nanoparticles. The abundance of microorganism in the environment and their great ability to adapt difficult conditions is prominent to use for metal reductions. So bacteria are a good choice for study, as they growing rapidly, inexpensive to cultivate and easy to manipulate and growth at different conditions such as temperature, oxygenation and incubation time. Lv et al [64] reported that changing the pH of the growth medium during incubation results in the production of nanoparticles of differing size and shape. Kalimuthu et al. [65] was proposed industrially significant intracellular AgNPs from the bacterium *Bacillus licheniformis* as it took only 24 hours to create AgNPs. However, also Shaverdi et al [66] reported the industrial bioproduction of AgNPs in 5 minutes by adding metal ions to a live culture, the cultures were centrifuged and the supernatant was tested for the ability to create metallic nanoparticles [66]. Due to the simplicity of purification and increase production rate by the extracellular type of formation so it is a more desirable [67]. An importing study by Sintubin et al.

Focused on the production of AgNPs by lactic acid bacteria. Many bacterial species were tested to synthesize AgNPs: *Lactobacillus* spp., *Pediococcus pentosaceus, Enterococcus faecium* and *Lactococcus garvieae* [68]. Many researchers reported green synthesis of metallic nanoparticles using plants or plant extracts [69]. In this field a huge, promising and effective result are observed for synthesis of Ag NPs. A study reported by Bar et al using plants for NPs production instead of bacteria or fungi to overcome pathogenicity with a simple green synthesis route for AgNPs from $AgNO_3$ salts using the extract from *Jatrophacurcas*. Results showed the production identical (10-20 nm) Ag NPs in 4 hours [70]. Plant extracts such as apiin (a glucoside compound) and leaf extract from magnolia, Persimmon, geranium, and Pine leaf have also been used as reducing agents of silver metal ions to produce silver nanoparticles [71, 72]. Another study, using *Acalypha indica* leaf extracts to produce Ag NPs with significantly homogenous size 20-30 nm. AgNPs were observed within 1 minute of exposure silver salt after using *Medicago sativa* seed exudates. In less than 50 minutes 90% Ag+ reduction occurred when the reaction was carried out at $30^{o}C$ [73]. Another plant that has the potential to reduce Ag+ is *Ocimum sanctum* [74]. The particles were spherical in shape, of 3-20 nm in size and a component of the leaf broth give to it stability [74]. Park et al use plant derived polysaccharides and phytochemicals to synthesis of gold and silver nanoparticles. They suggested that the use of such compounds can decrease the uses of toxic chemicals and greatest amounts of creating nanocomposites with different metals [75]. Soluble starch [76], chitosan [77], cellulose [78], dextran [79], alginic acid [80] and hyaluronic acid [81] have been used for the production of silver and gold nanoparticles successfully [75]. An extensive study was done by Song et al. on the production of AgNPs from a number of different plant leaf extracts. They examined the use of Pine, Persimmon, Ginkgo, Magnolia and Platanus extracts and compared their ability to produce AgNPs. They found that magnolia leaf broth is the best Ag+ reducer as it took only 11 minutes to reduce 90% of the Ag+ present in the sample [72]. Photocatalytic reduction [82], chemical reduction, photochemical or radiation-chemical reduction, metallic wire explosion, sonochemical, polyols, matrix chemistry [83], photoreduction [84], reverse micelle-based methods [10], and biologically synthesized [85]. Due to specific properties synthesis and production of NPs many authors give great receives attention to it so largely well understood specially the natural biological creation of NPs from certain microbes that are known to naturally synthesize particles which fit into the NP size range. The bacterium, *Pseudomonas stutzeri*, isolated from silver mines in Africa has been shown to have the ability to reduce Ag^+ ions and form AgNP of a well-defined size and a distinct morphology, ranging from completely spherical to triangular and hexagonal shapes. The author found that production of NP may occur within the periplasmic space of this bacterial species. Additionally, silver nanoparticles can be synthesized by several microorganisms such as the bacterial strains *Bacillus licheniformis, K. pneumoniae*, and fungi strains such as *Verticillium and Fusarium oxysporum, Aspergillus flavus* [86] Different forms of silver nanomaterials already in such products include: metallic silver nanoparticles [87], silver chloride particles [88], silver-impregnated zeolite powders and activated carbon materials [88], dendrimer–silver complexes and composites [89], polymer silver nanoparticle composites, silver-titanium dioxide composite nanopowders [90], and silver nanoparticles coated into polymers like polyurethane [91]. all of these forms of silver exert antimicrobial activity to some extent through release of silver ions , silver nanoparticles have been shown to be effective biocides against: (a) bacteria such as *Escherichia coli*, *Staphylococcus aureus, Staphylococcus epidermis, Leuconostoc mesenteroides, Bacillus subtilis, Klebsiella mobilis, and Klebsiella pneumoniae* among others [88, 92]; (b) fungi such as *Aspergillus niger, Candida albicans, Saccharomyces cerevisia, Trichophyton mentagrophytes, and Penicillium citrinum* [88, 93]; and (c) virus such as *Hepatitis B, HIV-1, syncytial* [94]. The mechanisms by which silver nanomaterials exert its antimicrobial activity for bacteria, commonly begin with the release of silver ions followed by generation of reactive oxygen species (ROS) [88] and cell membrane damage [72, 88]. NPs also have been studied as a medium for antibiotic delivery by the addition of various metal elements (especially silver and gold) for use in disinfectant filters [95], and coating various materials/surfaces as an antifouling biofilm [58, 69, 88] also detail the previous many commercial uses of silver as a biocide.

3. Application for uses of AgNP

The properties of AgNPs are potent as antibacterial activity (against both gram-positive & gram-negative bacteria), and also as being nontoxic toward mammalian cells. Maisch group demonstrated that it may be used in the future for inhibiting biofilm formation as to reduce the chances of microbial infections and rejection [96]. A recently designed synthesis of AgNPs and self-assemblies of amphiphilichydro regulators has immense implications in biomedicine, including organ transplantation and tissue engineering. This method of NPs are particularly efficient as potent bactericidal agents and have been found useful to prevent and control biofilm formation in food-contact surfaces, such as chopping boards and kitchen surfaces [97] because in the polymer matrix they facilitate efficient immobilization of the nanoparticles, and are they are more flexible to coating on surfaces of varying shapes and sizes, frequently reuse and monitoring through Sequential action cycles *In situ* synthesis and generation of metal nanoparticles within polymer films is a particularly efficient approach to such nanocomposite thin films [98]. But the antibacterial agent must be non-toxic to humans and also lethal against a wide spectrum of pathogens, cheap and easy to fabricate. One of the efficient and suitable ways to deploy the agent in a domestic setting would be as a coating on stirring devices. Safe drinking water plays a significant role in human health and well-being. About 1.8 million deaths and 61.9 million disability-adjusted life years (DALYs) worldwide registered by World Health Organisation (WHO) due to unsafe water, sanitation and poor hygiene. 99.8% of such deaths occur in developing countries, especially children ranking (90%) as the first victims [62]. At least one billion people worldwide have no chance to have clean drinkable water [99]. One of the basis causes of diarrheal diseases, as giardiasis, cholera, cryptosporidiosis and gastrointestinal infections is the contamination of groundwater and surface water sources with pathogenic bacteria such as *Escherichia coli* O157:H7, *Salmonella typhimurium*, *Shigella dysenteriae* and *Vibrio cholerae* continues to be one the major causes [100]. The 2010 updated report by the WHO and the United Nations Children’s Fund (UNICEF) reported that 884 million people in the world didn’t get drinking water from improved sources although tremendous progress has been made to date, Many areas lack the presence of clean water, most surface water are not safe for human consumption many different types of contaminant are present in untreated water such as pathogenic microorganism also to purify water from viral particles by NPs we can use silver as it enhance the photocatalytic activity of titanium dioxide [94], synthetic chemicals from petroleum and pharmaceuctals, heavy metals, pesticides, radioactive chemicals, to protect public health it is essential to treat drinking water this will require society to recycle water more effectively, and advanced technologies to remove chemical [101] and biological contaminates from water. Dispersion of chemicals in water as ozone, or strong oxidants containing halogens, peroxides, or related compounds that used through Common treatment methods for removing harmful biological species leave in water undesirable chemical byproducts in recent years the effective biocide silver nanoparticles (AgNPs) that have high specific surface area have been fabricated and tested [100]. They provide long term antibacterial effect without adverse chemical byproducts. But there is health and environmental hazards of nanoparticles related to the potential issues of aggregation during storage, difficult recovery once spread in the environment, and potential toxicity problems related to over-accumulation in the bodies of humans, animals, and plants [102]. That can be overcome for small-scale or point-of-use systems a functional nanomaterials have the potential to be used. AgNPs in particular, can be embedded in blotter papers and used to purify drinking water [93, 99] also by attachment of nano-particle (NPs) on enclosed in or attached to bigger structures such substrate. There is few devices which reported where AgNP in some cases may be loosely attached ceramic foams [64], polyurethane foam [91], cellulose papers [87], and other low cost filter materials [103] Our goal is to design a simple, light weight a powerful devices using silver nanoparticles of antibacterial properties attached it to the light weight porous substrates that can be linked in liquid cleansing devices used and that can be controlled electrically or thermally in Future. Several studies reported that AgNPs antibacterial activity is mainly on bacterial membranes caused their destroying probably due to the production of reactive oxygen species (ROS), including free radicals. Production of ROS is one of the primary mechanisms of nanoparticle toxicity as it destroys DNA, proteins, membranes as a result of oxidative stress, inflammation, [90]. The formation of pits in the *E. coli* cell wall can be confirmed by scanning and transmission electron microscopy images and also the accumulation of silver in bacterial membranes which increases permeability and therefore causing in cell death [104].

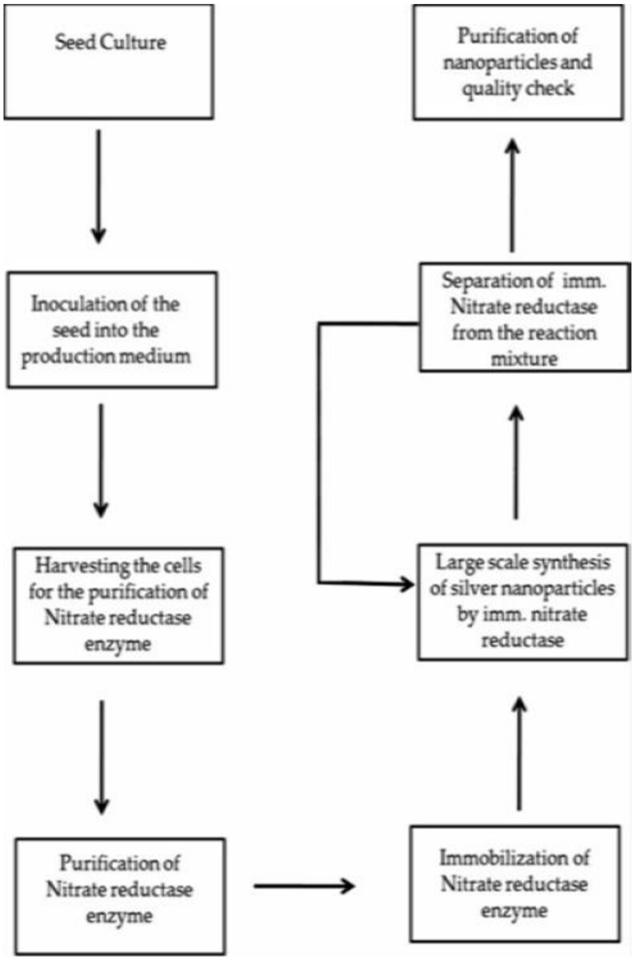

Fig. 2 An Insight into the Bacterial Biogenesis of Silver Nanoparticles, Process design for the industrial scale synthesis of silver nanoparticle by immobilizednitrate reductase

4. Conclusion

During the last decades, many efforts were put into the development of new green synthesis methods. Living organisms have huge potential for the production of nanomaterialsthat can be applied to many fields and more specifically to biomedicine. Organisms ranging from simple bacteria to highly complex eukaryotes can all be used for the production of nanoobjects with the desired size and shape. More recently, many investigations were carried out on

- synthesis of AgNPs-from the plant extract.
- Extracellular synthesis of silver nanoparticles from *E.coli* (Isolation and identification of *E. coli*, Antimicrobial susceptibility, Isolation and purification of plasmids then synthesis of AgNPs from *E. coli*.
- Intracellular synthesis of AgNPs from *B. subtilis*.

- Preparation of silver nanoparticles from *Pseudomonas aeruginosa* (Sample collection, Isolation of bacteria:, Fatty acid methyl esters, 16S rRNA Gene Sequence then Biosynthesis of AgNPs).
- Synthesis of Ag NPs stabilized by PVP at different concentration of metal salt.
- Evaluation of antimicrobial efficacy of AgNPs against selective pathogens.

The properties, processing, and integration of the NPs into various products are highly dependent upon NP morphology (size and shape) and their surface properties, which dictate both short- and long-range interactions (and possible organization) between particles and between the particles and the medium into which they are embedded. Non-biological methods for NP synthesis suffer from lack of control of morphology, the high energy input, and use of an array of toxic chemicals, so there is a definite need to investigate microbial methods of NP biosynthesis. Microbial metal resistance mechanisms appear to belinked to NP biosynthesis and microbes appear able to produce NPs of defined morphology. As understanding of metal resistance advances, this suggests potential for manipulating commercially relevant microbes for industrial scale synthesis of NPs. We conclude that NP biosynthesis a fertile field of microbiology-based research and that a clearer understanding of how to control and standardize growth and shape of these particles is needed. For technological applications, NPs have to be surface passivated (in order to be used for a variety of devices) and the possibility of using biological macromolecules as passivating agents open up many more research possibilities.

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