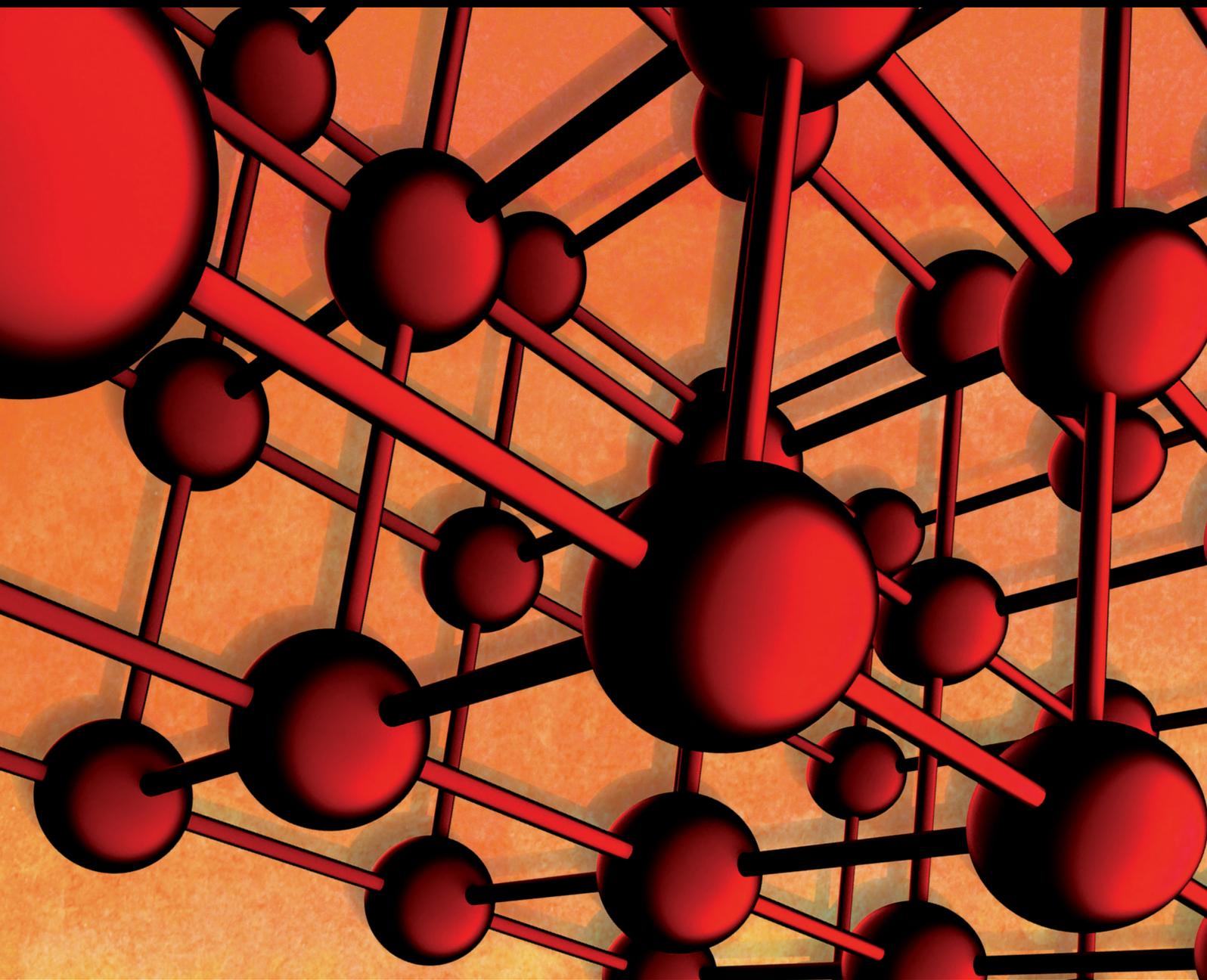


Advances in Materials Science and Engineering

# Silver Nanoparticles: Synthesis, Properties, and Applications

Guest Editors: Protima Rauwel, Erwan Rauwel, Stanislav Ferdov,  
and Mangala P. Singh





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## Editorial

# Silver Nanoparticles: Synthesis, Properties, and Applications

**Protima Rauwel,<sup>1,2</sup> Erwan Rauwel,<sup>3</sup> Stanislav Ferdov,<sup>4</sup> and Mangala P. Singh<sup>5,6</sup>**

<sup>1</sup>*Department of Physics, University of Oslo, 24 Sem Saelandsvei, 316 Oslo, Norway*

<sup>2</sup>*Institute of Physics, University of Tartu, Ravila 14c, 51014 Tartu, Estonia*

<sup>3</sup>*Tartu College, Tallinn University of Technology, Puiestee 78, 51008 Tartu, Estonia*

<sup>4</sup>*Department of Physics, University of Minho, 4800-058 Guimaraes, Portugal*

<sup>5</sup>*Department of Physics, Brock University, St. Catharines, ON, Canada L2S 3A1*

<sup>6</sup>*CWIT Capital Inc., 8 Baker Drive, St. Catharines, ON, Canada L2N 2V8*

Correspondence should be addressed to Protima Rauwel; [protima.rauwel@fys.uio.no](mailto:protima.rauwel@fys.uio.no)

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Silver nanoparticles have been of interest since the IV century AD owing to their dichroic character when integrated into glass. For over centuries, silver based compounds have been used as nontoxic, inorganic, and antibacterial agents owing to their biocidal properties in many applications such as wood preservatives or for water purification in hospitals. Nanomaterials demonstrate unique and significantly modified physical and chemical properties, compared to their macroscaled counterparts, which make them of particular interest. For this reason, silver nanoparticles have become of major interest for their antibacterial properties and are already integrated into applications such as wound treatment, sterilization, food sanitation, antibacterial textiles, and more recently drug delivery. In fact, silver nanoparticles exhibit a broad spectrum of antibactericidal and antifungicidal activities making them extremely popular in a diverse range of consumer products, including plastics, soaps, pastes, food, and textiles, thus increasing their market value. In the present context, they have attracted increasing interest due to their unique physical, chemical, and biological properties compared to their macroscaled counterparts. Silver nanoparticles are also studied by material scientists who investigate their integration into other materials in order to obtain enhanced properties, as, for example, in solar cells where silver nanoparticles are used as plasmonic light traps. These properties make them valuable in applications such as inks, microelectronics, medical imaging, and waste management. Traditionally, metal nanoparticles are produced by physical methods like ion sputtering or pulsed laser ablation and

chemical methods like solvothermal synthesis and sol-gel methods. More recently, however, environmentally friendly synthesis methods have been developed, called “green syntheses.” Depending on the chosen method, silver nanoparticles with different morphologies, sizes, and shapes can be obtained. Nevertheless, one of the critical criteria remains the size distribution that should be as narrow as possible to target specific applications.

The following special issue focuses on works that emphasize silver nanoparticles in today’s technologically advancing society. The contribution from K.-S. Chou and C.-H. Lee “Fabrication of Silver Interdigitated Electrode by a Stamp Method” proposes the fabrication of interdigitated electrodes via a stamp containing the imprint of the required electrode and employing silver ink. The stamping force required appears to be crucial in the reproducible printability of these electrodes. Further improvements in the printing can be obtained by changing the consistency of the silver ink, keeping in mind an ultimate automated method for such electrode fabrication. The method is attractive due to its cost-effectiveness and possible applications as sensors. On a different note, K.-H. Tseng et al. in their study entitled “A Study of Antibioactivity of Nanosilver Colloid and Silver Ion Solution” prepare the mood for a biomedical application of silver nanoparticles. Synthesis of the nanoparticles by nontoxic methods, such as electrical spark discharge without surfactants, is the appeal of the paper. Antibioactivity effectiveness of these ionic nanoparticles on yeast and bacteria is compared. Clearly, the study is of fundamental importance in

understanding the effect of silver ions on various bioactivities. In the same spirit, Y. Jeong et al. in "Assessment of Size-Dependent Antimicrobial and Cytotoxic Properties of Silver Nanoparticles" have studied not only antimicrobial activities of these silver nanoparticles but also their cytotoxicity. The size controlled synthesis of these nanoparticles further helped in assessing their size-dependent properties. Their work brings in new information on such materials as their experiments suggest that even though smaller nanoparticles tend to be more efficient antimicrobial agents, they also tend to present higher cytotoxicity for humans. In their work, they have studied antibacterial effect of Ag on *Methylobacterium* spp. and cytotoxicity on human peripheral blood mononuclear cells. They suggest that not only silver ions but also the nanosize of silver could be a factor in determining the antimicrobial activity.

The special issue also proposes review articles. The paper "Preparation of Silver Nanoparticles and Their Industrial and Biomedical Applications: A Comprehensive Review" by A. Haider I.-K. Kang, and as suggested by the title, is one such review article. The paper discusses the role of silver nanoparticles in industrial and biomedical applications. It describes various synthesis routes, including chemical and biological methods. Size of the nanoparticles and its effect on their cytotoxicity have been given a closer look along with their applications in textiles and plastic coatings, among others. Environmental consequences of releasing them into nature are also considered here. Furthermore, due to their antimicrobial properties, silver nanoparticles have also been an ingredient in wound dressings as presented by P. Uttayarat et al. in their research paper, "Radiolytic Synthesis of Colloidal Silver Nanoparticles for Antibacterial Wound Dressings." In this paper, the nanoparticles were synthesized by radiolysis where the precipitation of the silver nanoparticles depended on the gamma radiation dose. For a certain dose, the silver nanoparticles were efficient in inhibiting *Staphylococcus aureus* and *Pseudomonas aeruginosa*. They therefore prove to be good candidates as antiseptic agents in wound healing. Finally, a contribution from the guest editors entitled "A Review on the Green Synthesis of Silver Nanoparticles and Their Morphologies Studied via TEM" is part of this special issue. Since green synthesis is gaining importance and health and safety risks of other synthesis methods are continuously being assessed, the present review article by P. Rauwel et al. provides an overview of different green synthesis methods of silver nanoparticles using bacteria, fungi, yeast, and plant extracts. Furthermore, a review on the various particle sizes as a function of different plant extracts has been explored. In fact, even if organisms from bacteria to eukaryotes are used to produce silver nanoparticles, plant extracts possess the advantage of cost-effectiveness and abundance.

In conclusion, all the articles in the present issue present original research efforts in the field of the synthesis of silver nanoparticles, the study of their properties, and their integration in applications. This issue presents new research results developed in the field of interdigitated electrodes and their roles as antibacterial and antifungal agents. This issue also demonstrates the broad field of applications of silver nanoparticles via the two review articles that present the

recent advances in industrial and biomedical applications and the development of new environmentally friendly methods for the synthesis of silver nanoparticles. Finally, we hope that this issue is useful and a reference for scientists, researchers, and teachers in the field of materials science and nanotechnology.

Protima Rauwel  
Erwan Rauwel  
Stanislav Ferdov  
Mangala P. Singh

## Review Article

# A Review on the Green Synthesis of Silver Nanoparticles and Their Morphologies Studied via TEM

Protima Rauwel,<sup>1</sup> Siim Küünal,<sup>2</sup> Stanislav Ferdov,<sup>3</sup> and Erwan Rauwel<sup>1,2</sup>

<sup>1</sup> Department of Physics, University of Tartu, Riia 142, 51014 Tartu, Estonia

<sup>2</sup> Tartu College, Tallinn University of Technology, Puiestee 78, 51008 Tartu, Estonia

<sup>3</sup> Department of Physics, University of Minho, 4800-058 Guimarães, Portugal

Correspondence should be addressed to Erwan Rauwel; [erwan.rauwel@ttu.ee](mailto:erwan.rauwel@ttu.ee)

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Silver has been recognized as a nontoxic, safe inorganic antibacterial/antifungal agent used for centuries. Silver demonstrates a very high potential in a wide range of biological applications, more particularly in the form of nanoparticles. Environmentally friendly synthesis methods are becoming more and more popular in chemistry and chemical technologies and the need for ecological methods of synthesis is increasing; the aim is to reduce polluting reaction by-products. Another important advantage of green synthesis methods lies in its cost-effectiveness and in the abundance of raw materials. During the last five years, many efforts were put into developing new greener and cheaper methods for the synthesis of nanoparticles. The cost decrease and less harmful synthesis methods have been the motivation in comparison to other synthesis techniques where harmful reductive organic species produce hazardous by-products. This environment-friendly aspect has now become a major social issue and is instrumental in combatting environmental pollution through reduction or elimination of hazardous materials. This review describes a brief overview of the research on green synthesis of silver metal nanoparticles and the influence of the method on their size and morphology.

## 1. Introduction

For over centuries, silver based compounds were used as non-toxic inorganic antibacterial agents owing to their biocidal properties in many applications such as wood preservatives, water purification in hospitals, in wound or burn dressing, and so forth. In fact, silver ions and their related compounds have low toxicity toward animal cells but present a high toxicity to microorganisms like bacteria and fungi. The recent advances in the field of nanoparticle synthesis have a strong impact in many scientific areas and the synthesis of silver nanoparticles has also followed this tendency. These unique properties of nanomaterials have spurred numerous investigations and applications in electronics, nanomedicine, biomaterials, energy, and food. In fact, silver based compounds are much cheaper than gold based one; moreover, silver nanoparticles are now considered as an important class of nanomaterials. They are presently mainly used as catalyst [1] or antibacterial/antifungal agents [2].

Environmentally friendly synthesis methods are becoming more and more popular in chemistry and chemical technologies. This trend has several origins, including the need for greener methods counteracting the higher costs and higher energy requirements of physical and chemical processes. For this reason, scientists search for cheaper methods of synthesis. The other reason is that conventional methods for nanoparticle synthesis usually require harmful reductants such as sodium borohydride or hydrazine and many steps in the synthesis procedure including heat treatments, often producing hazardous by-products. In order to reduce the environmental impact of nanoparticle synthesis, greener routes have been investigated for over a decade. The principles of green chemistry were presented by Anastas and Warner who developed 12 principles that eloquently describe green chemistry [3]. Green chemistry should aim at thwarting waste, minimizing energy use, employing renewable materials, and applying methods that minimize risk. The three main concepts for the preparation of nanoparticles

in a green synthesis approach are the choice of the solvent medium (preferably water), an environmentally friendly reducing agent, and a nontoxic material for the stabilization of the nanoparticles [4].

To be energy efficient, the synthesis processes should be carried out close to ambient temperature and pressure and under neutral pH. The biological systems then appear as the most suitable factory for reaching such natural chemistry conditions. It is well known that many microorganisms can provide inorganic materials either intra- or extracellularly [5] and it was found that some of these microorganisms can be used as ecofriendly nanofactories for the production of nanomaterials, more particularly for the production of silver metal nanoparticles (Ag NPs).

Many approaches were investigated, and microorganisms such as bacteria, yeasts, fungi, and algae were used in the biosynthesis of metal nanoparticles. More recently, the utilization of plants for the production of metal nanoparticles has spurred numerous investigations in the field of green synthesis. The aim of this review is to provide a brief overview of the current research on the green synthesis of silver nanoparticles and describe how the different methods of synthesis can affect the size and the morphologies of the silver metal nanoparticles.

## 2. Green Synthesis Methods

**2.1. Green Synthesis Using Bacteria as a Medium.** Bacteria are known to produce inorganic materials either intra- or extracellularly. This makes them potential biofactories for the synthesis of nanoparticles like gold and silver. Silver is well known for its biocidal properties; however, some bacteria are known to be silver resistant [6] and can accumulate silver on the cell wall to as much as 25% of their dry weight biomass, thus suggesting their use in industrial recovery of silver from ore materials [7]. Therefore, the use of prokaryotic bacteria as nanofactories was first studied. First noble metal nanoparticle synthesis, using bacteria, was done using silver resistant bacterial strains *Pseudomonas stutzeri* AG259, which were cultured in high concentrations of silver nitrates. It was demonstrated that the cells accumulate silver in large quantities and the majority of the silver was deposited in the form of particles of 200 nanometers of diameter [8]. Significant results were observed when bacteria *Proteus mirabilis* PTCC 1710 were used for producing silver nanoparticles. It was found that depending on the type of “broth” used during the incubation of bacteria, extracellular or intracellular synthesis can be promoted. This kind of selection makes bacteria-based green synthesis flexible, inexpensive, and a suitable method for large-scale production [9]. It is important to point out that bacteria continued to grow after the synthesis of silver nanoparticles. However, the main drawback of using bacteria as nanofactories is the slow synthesis rate and the limited number of sizes and shapes available compared to the conventional chemical methods of synthesis. For this reason, fungi-based nanofactories and chemical reaction involving plant based materials were investigated (see Table 1) [10].

**2.2. Green Synthesis Using Fungi as Medium.** Similar to bacteria, due to their tolerance and metal bioaccumulation ability, high binding capacity, and intracellular uptake, fungi have been of interest in biological production of the metallic nanoparticles [11]. Compared to bacteria, fungi are simpler to handle in a laboratory process. The mechanism of nanoparticle production using fungi is different; fungi secrete large amounts of enzymes which are used to reduce silver ions that induce the formation of the metal nanoparticles [12].

The first synthesis involving fungus-mediated approaches for the metal nanoparticle synthesis was performed in the beginning of the 20th century, and Ag NPs with diameter of  $25 \pm 12$  nm were synthesized using fungus *Verticillium* [13, 14]. In previous studies involving bacteria, bacteria *Pseudomonas stutzeri* AG259 isolated from silver mines were able to produce Ag NPs of well-defined size and distinct morphology within the periplasmic space of the bacteria [8]. Synthesis using *Verticillium* takes the green approach even further. During the exposure of the fungus to AgNO<sub>3</sub> solution, the reduction of ions and the formation of Ag NPs take place. Nanoparticles were approximately 25 nm in diameter presenting a rather good monodispersity and spherical morphology. Contrary to bacteria, Ag NPs were formed below the surface of the fungal cells [13]. This result differs from the work of Klaus et al. 1999, where particle morphologies synthesized using bacteria ranged from spherical, triangular to hexagonal. The mechanism of nanoparticle formation was then studied and the main hypothesis is that, in the case of fungi-based synthesis, the NPs are formed on the surface of the mycelia and not in the solution. It was then suggested that in the first step Ag<sup>+</sup> ions are adsorbed on the surface of the fungal cells due to electrostatic interaction between negatively charged carboxylate groups in enzymes present in the cell wall of mycelia and positively charged Ag ions. Finally, the silver ions are then reduced by the enzymes present in cell wall, leading to the formation of silver nuclei [13]. The shift from bacteria to fungi as a means of developing natural nanofactories offers the advantages of simpler downstream processing and handling of the biomass. Compared to bacteria, fungi are known to secrete much higher amounts of proteins, which tends to significantly increase the productivity of this biosynthetic approach; moreover, fungi could be used for the production of large amounts of metal nanoparticles.

The first report involving extracellular synthesis of silver nanoparticles using eukaryotic systems such as fungi was reported by Ahmad et al. 2003. They showed that secreted enzymes are responsible in the reduction process [15]. Before this report, all the fungi based biosyntheses were intracellular. Extracellular synthesis is advantageous as the synthesized nanoparticles will not bind to the biomass [16, 17] and it is therefore possible to extend this approach for the biosynthesis of nanomaterials over a range of chemical compositions, such as oxides, nitrides, and so forth.

When compared to other classes of microorganisms, their ecofriendliness and simplicity during handling lead to increasing the use of fungi in green synthesis. For example, fungus like white rot fungus is nonpathogenic and this contributes to the mass production of Ag NPs [18]. Another

TABLE 1: Size dependent synthesis of silver nanoparticles via eco-friendly synthesis routes.

Type	Diameter	References
Bacteria		
<i>Pseudomonas stutzeri</i> AG259	200 nm	[8]
<i>Proteus mirabilis</i> PTCC 1710	10–20 nm	[9]
Fungi		
<i>Verticillium</i>	25 nm ± 12 nm	[13, 14]
<i>Fusarium oxysporum</i>	5–15 nm	[15]
<i>Cladosporium cladosporioides</i>	10–100 nm	[16]
<i>Fusarium oxysporum</i>	20–50 nm	[17]
<i>Aspergillus fumigatus</i>	5–25 nm	[19]
<i>A. flavus</i>	5–30 nm	[20]
Yeast		
MKY3	2–5 nm	[23]
Baker's yeast, <i>Saccharomyces cerevisiae</i>	60–80 nm	[24]
Algae		
<i>Sargassum wightii</i>	8–12 nm	[25]
Plant extracts		
Alfalfa sprouts	2–20 nm	[26]
<i>Geranium</i> leaves	14–46 nm	[27]
<i>Chrysanthemum indicum</i> L.	38–72 nm	[38]
<i>Acacia leucophloea</i>	17–29 nm	[39]
<i>Ganoderma neojaponicum</i>	5 nm	[40]
<i>Imazeki</i>		
<i>Colletotrichum</i> sp.	20–40 nm	[41]
Caffeine and tea extract	60 nm	[42]
Gelatin and glucose	3.68 nm and 5.28 nm	[43]
<i>Thevetia peruviana</i> (latex)	10–60 nm	[45]
<i>Elaeagnus latifolia</i>	30–50 nm	[46]
<i>Leptadenia reticulata</i>	50–70 nm	[47]
Olive extract (1 mL)	30 nm	[48]
Olive extract (5 mL)	15 nm	[48]
<i>Terminalia chebula</i> fruit extract	100 nm	[49]
<i>Tinospora cordifolia</i>	17–29 nm	[50]

important factor for choosing the method of synthesis is the reaction rate. First report of rapid synthesis using fungi was using *Aspergillus fumigatus* that allowed obtaining monodispersed Ag NPs within 10 minutes [19]. In addition, one of the most common molds *Aspergillus fumigatus* was used to make Ag NPs in a matter of minutes, when silver ions entered into contact with the cell filtrate. These investigations were clear examples describing suitability and the potential of using fungi for mass production of nanoparticles. More recently, Ag NPs were synthesized using *A. Flavus* fungi to be combined with antibiotics to enhance the biocidal efficiency against multidrug-resistant bacteria. This study demonstrated the efficiency of antibiotics combined with Ag NPs [20].

Similar to fungi, yeasts were also widely investigated for silver nanoparticle synthesis [12, 21, 22]. Silver-tolerant yeast strain MKY3 was first used for extracellular synthesis. The outcome of the synthesis was satisfying due to simplicity of the separation of the nanoparticles when using differential thawing [23]. After that, several studies followed but until recently synthesis has never been carried out by commercial baker's yeast available in grocery stores. All the aggravating steps of cultivation of the yeast were avoided, thus making the process much simpler [24].

Similar to moving from prokaryotes to eukaryotes green synthesis, the utilization of eukaryotic autotrophs widened the possibilities of green synthesis. For example, using marine algae *Sargassum wightii* allowed obtaining very stable nanoparticles compared to other biological methods [25].

**2.3. Green Synthesis Using Plants and Plant Extract as a Medium.** One of the first approaches of using plants as a source for the synthesis of metallic nanoparticles was with alfalfa sprouts [26], which was the first report on the formation of Ag NPs using a living plant system. Alfalfa roots have the capability of absorbing Ag from agar medium and transferring them into the shoots of the plant in the same oxidation state. In the shoots, these Ag atoms arranged themselves to form nanoparticles by joining themselves and forming larger arrangements. In comparison to bacteria and fungi, green synthesis using plants appears to be faster and the first investigations demonstrate that synthesis procedures are able to produce quite rapidly Ag NPs. Shankar et al. showed that using *Geranium* leaf takes around nine hours reaching 90% reaction compared to the 24 to 124 hours necessary for other reactions reported earlier [27]. Therefore, the use of plant extracts in green synthesis has spurred numerous investigations and studies up till now. It was demonstrated that the production of metal nanoparticles using plant extracts could be completed in the metal salt solution within minutes at room temperature, depending on the nature of the plant extract. After the choice of the plant extract, the main affecting parameters are the concentration of the extract, the metal salt, the temperature, the pH, and the contact time [28].

In addition to the synthesis parameters, the main issue is the choice of the plant from which the extract could be used. The advantages of using plants for the synthesis of nanoparticles are that the plants are easily available and safe to handle and possess a large variety of active agents that can promote the reduction of silver ions. Most of the plant parts like leaves, roots, latex, bark, stem, and seeds are being used for nanoparticle synthesis [10]. The most important point is the active agent contained in these parts which makes the reduction and stabilization possible. Ecofriendly plant extracts contain biomolecules, which act as both reducing and capping agents that form stable and shape-controlled nanoparticles. Main compounds which affect the reduction and the capping of the nanoparticles are biomolecules such as phenolics, terpenoids, polysaccharides, flavones, alkaloids, proteins, enzymes, amino acids, and alcoholic compounds. However, quinol and chlorophyll pigments, linalool, methyl chavicol, eugenol, caffeine, theophylline, ascorbic acid, and

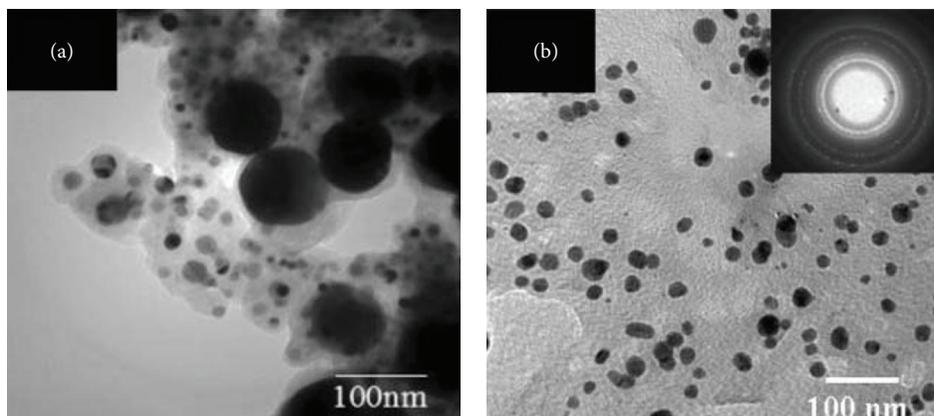


FIGURE 1: Ag NPs from coffee (a) and tea (b) extract [42]. Copyright license number: 3482980509111.

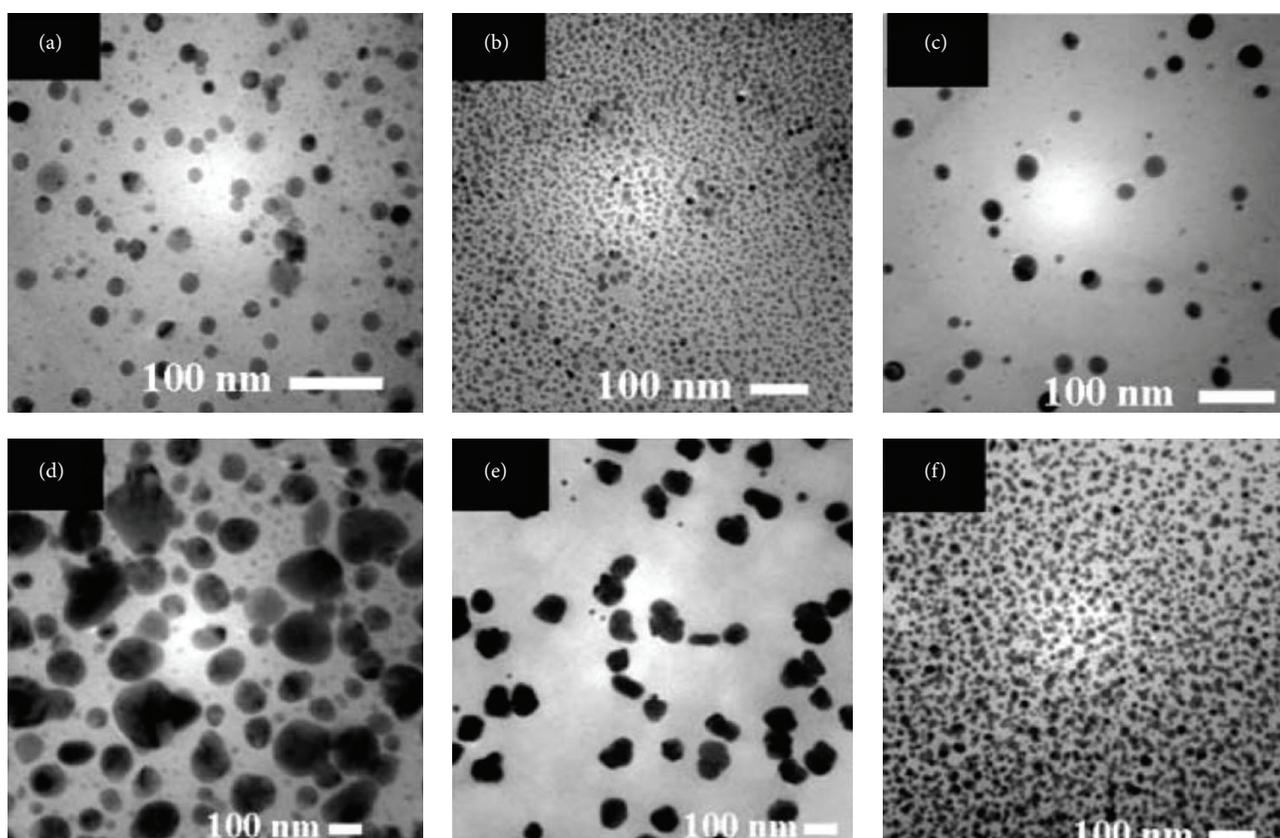


FIGURE 2: TEM image of silver nanoparticles synthesized using (a) Bigelow tea, (b) Folgers coffee, (c) Lipton tea, (d) Luzianne tea, (e) Sanka coffee, and (f) Starbucks coffee extract at room temperature in one step without using any hazardous reducing chemicals or nondegradable capping agents [42]. Copyright license number: 3482980509111.

other vitamins have also been reported [29–36]. The non-toxic phytochemicals including aforementioned flavonoids and phenols have unique chemical power to reduce and also effectively wrap nanoparticles, thus preventing their agglomeration. Phenolic compounds possess hydroxyl and carboxyl groups, which are able to bind to metals [37].

Most of the AgNPs synthesized via green synthesis are investigated for biomedicine and more particularly as

antibacterial agent or for cancer treatment. Recent reports showed that it was possible using *Chrysanthemum indicum* L. [38] or *Acacia leucophloea* extract [39] to synthesize Ag NPs of diameter ranging from 38–72 nm to 17–29 nm, respectively. Both samples demonstrated very good antibacterial properties. In the same manner, *Ganoderma neojaponicum* Imazeki was used for the synthesis of Ag NPs as potential cytotoxic agents against breast cancer cells [40]. As these methods

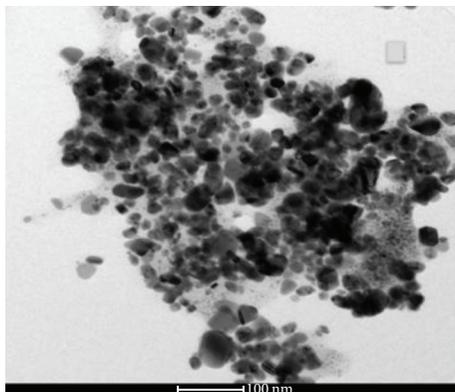


FIGURE 3: Representative TEM image of Ag NPs synthesized at room temperature using latex extract from the stems of fresh fruits of *Thevetia peruviana* [45] (reproduced with permission from IOP).

Involving green chemistry are being more and more explored and scientists are starting to combine different options together, it was recently reported that the symbiotic biological systems such as *Geranium* leaf combined with endophytic fungus *Colletotrichum* sp. can synergize the outcome of the reaction. In fact, plants contain biomolecules which are able to stabilize unstable particles whereas fungi secrete enzymes for reduction [41].

### 3. Morphological Characterization via Transmission Electron Microscopy

The size distribution of nanoparticles in general is an important issue as nanoparticles exhibit different physical and chemical properties depending on their shape and size. Synthesis methods that generate uniformly sized and shaped nanoparticles are therefore being pursued. Transmission electron microscopy (TEM) is therefore one of the most adapted techniques to study the size and shape of the nanoparticles and provide their distribution. It is important to note that the majority of the TEM studies were performed on plant extracted green synthesis of silver nanoparticles.

Many different plant extracts have been used to prepare silver nanoparticles in the aim of producing Ag NPs presenting different capping layer molecules and presenting different morphologies. These studies in TEM have shown that the presence of a capping layer in plant mediated synthesis of Ag NPs, where the plant extract acts as capping layers, shapes the nanoparticle during its growth. It also has an effect on the size distribution of these nanoparticles. The use of medicinal plants in the synthesis of Ag NPs is not only used for size and shape control but also used to provide properties to the Ag NPs along with the antimicrobial properties of the plant. In this section, special emphasis is given to the Ag NPs morphology and size distribution as a result of synthesis parameters using TEM.

Everyday products such as tea and coffee can be used to produce Ag NPs, namely, tea leaves and coffee beans. Nadagouda et al. have explained that the caffeine and polyphenol in tea and coffee help in forming complexes with

metal ions and reducing them to the corresponding metal [42]. They have used commercially available coffee and tea extract mixed with  $\text{AgNO}_3$ . TEM samples were prepared by diluting the nanoparticles in water. The TEM images provide monodispersed nanoparticles in each case, indicating that the polyphenols act not only as a reducing agent but also as a capping agent and therefore restrict their growth to 60 nm (Figure 1). In Figure 1, the TEM images provide size distribution of Ag NPs and indicate that tea extract reduced Ag NPs present a small particle size distribution compared to the caffeine reduced ones (Figure 1(b)). By using different types of tea and coffee extract the authors have shown that the size of the nanoparticles can be further varied (Figure 2).

Other edible products such as gelatin and glucose have also been employed by Darroudi et al. to modify the Ag NPs size and shape [43]. The synthesis was carried out by dissolving gelatin in water and adding silver solution. A small amount of glucose was also added and the solution was heat treated at different temperatures, that is, 20, 40, and 60°C. Synthesis with and without the presence of glucose was performed. The particle size showed a decrease when the temperature was increased and when glucose was absent. At 60°C, the nanoparticles had an average size of 3.68 nm and 5.28 nm. This is attributed to the rate of reduction reaction of  $\text{AgNO}_3$ .

In another study, ecofriendly honey was used as a reducing agent and replaced synthetic reducing agents such as hydrazine and dimethyl formamide which are not totally environmentally safe. In this study, the particle size depended on the concentration of honey and the pH of the aqueous solution [44]. Another encounter where Rupiahsi et al. used latex in the green synthesis of Ag NPs has also been reported [45]. Milky white latex was extracted from the stems of fresh fruits of *Thevetia peruviana* and filtered out. This was then mixed with  $\text{AgNO}_3$ . The TEM images of the particles thus obtained show spherical particles with a wide size distribution with 75% of the nanoparticles presenting particle size between 10 and 30 nm (Figure 3). Less than 10% of the nanoparticles were under 10 nm in size and between 50 and 60 nm. Most of the particles in the TEM image are polydispersed despite latex being used as a capping and reducing agent; the nanoparticles were nevertheless spherically shaped. However, other nanoparticle shapes of Ag NPs are also possible with other green synthesis methods as explained below.

Another plant mediated synthesis of Ag nanoparticles includes *Elaeagnus latifolia* a native evergreen shrub to Asia. In the work of Phanjom et al. the nanoparticles were precipitated after a room temperature reaction of the leaf extract with  $\text{AgNO}_3$  and provided a size distribution of Ag NPs between 30 and 50 nm (Figure 4) [46]. However, the TEM contrasts exhibit the presence of defects such as stacking faults or twinning in them (Figure 4). The authors also suggest the formation of an organic capping layer on the surface of these nanoparticles thereby explaining the high level of monodispersion of the nanoparticles.

Swamy et al. have employed *Leptadenia reticulata* which is a medicinal plant native to the Indian subcontinent [47]. It has been actively used to alleviate symptoms of

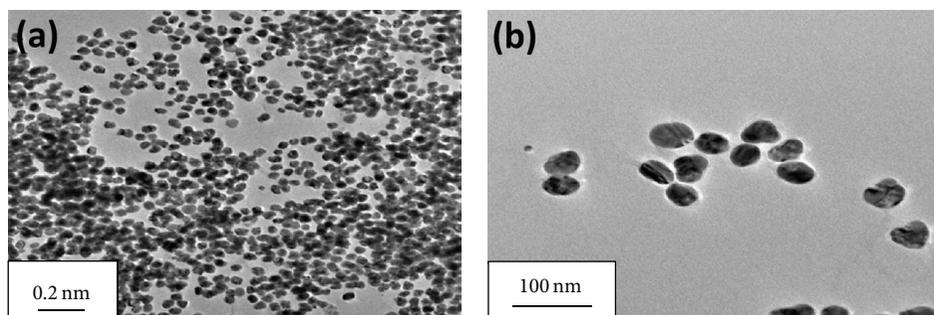


FIGURE 4: TEM images of AgNPs synthesized with *Elaeagnus Latifolia* as a reducing agent: (a) overview of AgNPs and (b) higher magnification image of the Ag NPs showing defects [46] (reproduced with permission from the journal).

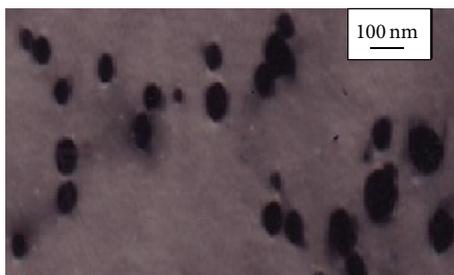


FIGURE 5: Ag NPs synthesized using *Leptadenia reticulata* extract [47] (reproduced with permission from Springer).

hematopoiesis, emaciation, cough, dyspnoea, fever, burning sensation, and night blindness. Extracts of this plant are also used to cure skin disorders. For the synthesis, once again  $\text{AgNO}_3$  was mixed with the leaf extract in 1:9 proportions. The nanoparticles obtained were stored in ionized water and frozen for further study. For TEM study these nanoparticles were suspended on a carbon coated copper grid. The particles appear oblong with sizes between 50 and 70 nm (Figure 5).

Olive leaf extract with  $\text{AgNO}_3$  has been used by Khalil et al. for the synthesis of Ag NPs and their antibacterial properties have been evaluated [48]. It is well known that olive leaf is effective against *Staphylococcus aureus*, *Pseudomonas aeruginosa*, and *Escherichia coli*. Their studies focused first of all on the effect of concentration of the leaf extract on the particle size. In this context they observed a decrease in the particles size with increase in the olive leaf extract concentration during reaction as indicated in Figure 6. The authors have calculated that for the 1 mL concentration average particle size was 30 nm (Figure 6) while for 5 mL of olive leaf extract concentration the particle size was less than 15 nm (Figure 6).

Moreover, the effect of pH of the reaction solution on the particles size was also evaluated and they noticed a clear decrease in particle size with increase in pH. Here Ag NPs with 2 different pH values (Figure 7) were studied with TEM and smaller particle sizes were obtained for higher pH (Figure 7). It is therefore possible to vary the pH and the extraction concentration and tailor the size of the NPs.

*Terminalia chebula* fruit extract has also been used by Kiran Kumar et al. for the production of silver nanoparticles [49]. In their reaction  $\text{Ag}_2\text{SO}_4$  was mixed with the extraction liquid compared to other methods presented here where  $\text{AgNO}_3$  was used.

The nanoparticles produced by this method showed sharp facets. All the nanoparticles were under 100 nm in size. The shape anisotropy is believed to be due to lack of protective biomolecules which assists in the homogeneity of the shape during growth, thus forcing them to attain thermodynamic stability by acquiring shapes such as triangles and hexagons. The monodispersed aspect of these nanoparticles was again attributed to the capping layer of polyphenols which are known to reduce  $\text{Ag}^{+2}$  to  $\text{Ag}^{+0}$  and the oxidized polyphenol binds to the Ag NPs via  $-\text{C}=\text{O}$  bonds and also simultaneously stabilizes them.

Other plant mediated Ag NPs synthesis involves *Tinospora Cordifolia* by Anuj and Ishnava [50]. Here they used the stem extract to obtain a suspension that was then mixed with  $\text{AgNO}_3$ . The nanoparticles produced in their study were spherical except for larger particles that presented nonuniform shapes. They also noticed a fine film on the TEM grid which corresponded to the capping layer produced from the *Tinospora Cordifolia* extract (Figure 8).

More recently, it was shown that Ag NPs synthesized using *Acacia Leucophloea* extract present a spherical morphology with a diameter ranging from 17 to 29 nm. The authors observed an enhancement of the antibacterial activity of Ag NPs synthesized using this method [39].

#### 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 nanomaterials that 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. Prokaryotes are the simplest forms of biomass and therefore are easy to manipulate genetically to make them produce more desired substances for synthesis. However, the cultivation of the bacteria and large scale production remains problematic

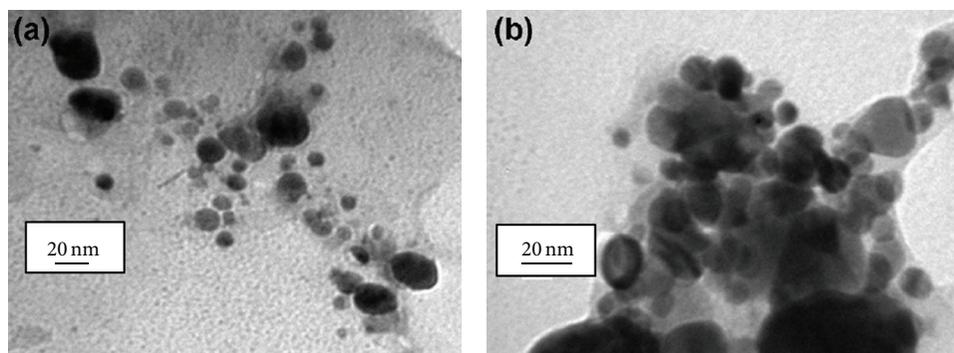


FIGURE 6: TEM micrographs with (a) 1 mL and (b) 5 mL olive leaf extract [48] (reproduced with permission from the journal).

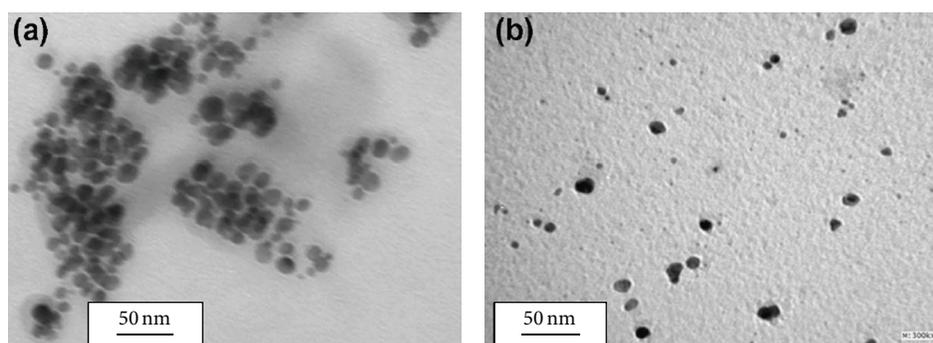


FIGURE 7: TEM micrograph of the silver nanoparticles [48]: (a) at pH 3, nm and (b) at pH 8 (reproduced with permission from the journal).

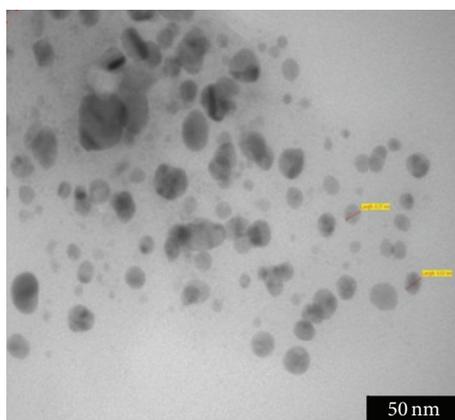


FIGURE 8: Ag NPs synthesized using *Tinospora Cordifolia* extract as a reducing agent [50] (reproduced with permission from the journal).

compared to others. Therefore, as a first approach, bacteria were studied as first nanofactories for the production of noble metal nanoparticles. However, the low synthesis rate and the limited number of size and shape distributions available oriented the investigations to the use of fungi and algae.

Fungi present a suitable option for large-scale green nanoproduction. They are easy to handle during downstream processing and they secrete large amounts of enzymes needed in the reduction. They also present filamentous tolerance towards metals, high binding capacity, and intracellular

uptake. Nevertheless, the genetic manipulation to overexpress specific enzymes in order to intensify synthesis is much more difficult among eukaryotes.

More recently, many investigations were carried out on the possible use of plant extracts and the number of research papers published in this field has increased exponentially during the last 2 years due to their easy availability, environmental friendliness, and cost-effectiveness. Moreover, plants contain the most effective compounds for synthesis and therefore enhance the synthesis rate. Size and shape distribution of the nanoparticles as obtained from TEM studies shows that many factors affect their morphologies including the nature of the plant extract, the pH of the solution, and the temperature of the reaction. Nevertheless, obtaining uniform size and shape distribution of Ag NPs remains a subject of investigation.

### Conflict of Interests

The authors declare that there is no conflict of interests regarding the publication of this paper.

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## Research Article

# Radiolytic Synthesis of Colloidal Silver Nanoparticles for Antibacterial Wound Dressings

**Pimpon Uttayarat, Jarurattana Eamsiri,  
Theeranan Tangthong, and Phiriyatorn Suwanmala**

*Thailand Institute of Nuclear Technology (Public Organization), 9/9 Moo 7, Tambon Saimoon,  
Ongkarak, Nakorn Nayok 26120, Thailand*

Correspondence should be addressed to Pimpon Uttayarat; [puttayar@alumni.upenn.edu](mailto:puttayar@alumni.upenn.edu)

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Radiolytic synthesis provides a convenient and environmentally-friendly approach to prepare metallic nanoparticles in large scale with narrow size distribution. In this report, colloidal silver nanoparticles (AgNPs) were synthesized by gamma radiation using poly(vinyl alcohol) (PVA) or silk fibroin (SF) as stabilizers and were evaluated for their antibacterial properties. The conversion of metallic silver ions to silver atoms depended on irradiation dose and stabilizer concentration as determined by UV-Vis spectrophotometry and transmission electron microscopy. The uniformly dispersed AgNPs with diameter  $32.3 \pm 4.40$  nm were evaluated as antiseptic agents in films composed of chitosan, SF, and PVA that were processed by irradiation-induced crosslinking. Using disc diffusion assay, the films containing 432 ppm AgNPs could effectively inhibit the growth of both *Staphylococcus aureus* and *Pseudomonas aeruginosa*. Therefore, we have demonstrated in our present study that gamma radiation technique can potentially be applied in the mass production of antibacterial wound dressings.

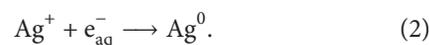
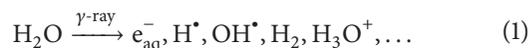
## 1. Introduction

Since ancient times, silver has been recognized for its medicinal use in wound treatment as well as its hygienic use in food and water preservation [1, 2]. During the past few decades, the antibacterial properties of silver become the center of research interest as several investigations have demonstrated the potent toxicity of silver against various Gram-positive and Gram-negative bacterial strains [1–7]. This allows silver to be used in a wide range of biomedical applications such as prevention of infection, wound healing, and anti-inflammation. A recent study also reported the ability of silver to synergistically enhance bactericidal activity of antibiotics against drug-resistant bacteria as well as biofilms in which the latter have become clinical challenges in treating chronic infections [2].

The advance in nanotechnology has driven the development of silver particles with nanometer size scale that yields many interesting properties that range from electronics to biomedical applications. The key synthesis aspects of silver nanoparticles (AgNPs) focus on the ability to control their size, shape, and dispersity [8]. Colloidal AgNPs can be

prepared by the reduction of silver ion ( $\text{Ag}^+$ ) precursor to their zero-valent ( $\text{Ag}^0$ ) nuclei, which are stabilized by surfactants or polymers during the growth process to prevent agglomeration of large particles [3, 5, 8–10]. Various techniques have been reported in the synthesis of AgNPs including light-assisted reduction of  $\text{Ag}^+$  by gamma radiation, UV radiation, laser ablation, and chemical reduction by reducing agents such as sodium borohydride, hydrazine, formaldehyde, ascorbic acid, and glucose [8–10]. Among these techniques, radiolytic synthesis by gamma radiation provides a major advantage for a large-scale production of monodisperse particles as it generates a uniform distribution of reducing agents in the entire solution during the irradiation process [9].

In the radiolytic synthesis of AgNPs, which was first developed by Henglein and Tausch-Treml [11], radiolysis of water generates active species such as hydrated electrons and hydroxyl radicals [9–12]:



Hydrated electrons directly reduce  $\text{Ag}^+$  precursor to  $\text{Ag}^0$  nuclei, which progressively coalesce into clusters. To prevent hydroxyl radicals from oxidizing nascent  $\text{Ag}^0$  clusters, scavengers such as alcohols are usually added prior to the irradiation process [9, 10] to react with the hydroxyl radicals. This results in the formation of hydroxyalkyl radicals which in turn react with other molecules in solution to produce new radicals for the reduction of  $\text{Ag}^+$  to  $\text{Ag}^0$  [9].

To ensure the dispersion of AgNPs in aqueous solution, polymers containing functional groups such as  $-\text{NH}_2$ ,  $-\text{COOH}$ , and  $-\text{OH}$  are added as stabilizers during the irradiation process [5, 12]. Due to their high affinity for silver, these polymers anchor on the surface of AgNPs, providing electrostatic repulsion and steric hindrance [12] that prevent rapid agglomeration of small clusters into large particles. Previous studies have reported the use of both natural and synthetic polymers such as chitosan, gelatin, carboxymethyl cellulose, poly(vinyl alcohol) (PVA), and polyvinylpyrrolidone (PVP) as stabilizers [3, 5, 10]. The resulting AgNPs are shown to be monodisperse with size that ranged from a few to tens of nanometers [3, 5, 10, 12, 13].

In biomedical application, wound dressings possessing hydrogel and antimicrobial properties are desirable as coverage over wounds to absorb exudates and prevent bacterial infection, thus promoting the regeneration of skin tissue and wound healing [7, 14]. Naturally derived polymers such as silk fibroin (SF) from cocoons of silkworms and chitosan (CS) from chitin of crustaceans have recently attracted considerable attention as a choice of materials for the fabrication of wound dressings due to their biocompatibility, water absorption, and excellent mechanical properties [15–17]. In this report, colloidal AgNPs were prepared by radiolytic synthesis and evaluated as an antiseptic agent when incorporated into dressing films. In the irradiation-induced reduction process, we tested SF protein as stabilizer in comparison to PVA. The morphology and dispersion of nanoparticles were shown to depend on irradiation dose and stabilizer concentration. Disc diffusion assay showed that SF-CS-PVA films embedded with AgNPs could effectively inhibit bacterial growth.

## 2. Materials and Method

**2.1. Radiolytic Synthesis of Colloidal AgNPs.** Silver nitrate ( $\text{AgNO}_3$ , Merck, Germany) was used as the starting source of silver for the radiolytic-induced reduction of  $\text{Ag}^+$  to  $\text{Ag}^0$ . Commercial PVA ( $M_w$  72,000, Merck, Germany) and SF protein (molecular weight  $\sim 20$ –238 kDa) extracted from cocoons of silkworm *Bombyx mori* using the previously described method [7] were used as stabilizers. Reversed osmosis water was used to prepare solutions in all experiments. Solutions containing 40 mM  $\text{AgNO}_3$  were prepared in 0.5, 1, and 2% (w/v) PVA ( $M_w$  72,000, Merck, Germany) with 0.8 M ethanol or in 0.5, 1, 2, and 5% (w/v) SF. After deaeration by bubbling with nitrogen gas, the solutions were irradiated by gamma rays from a Co-60 source (gammacell 220) at doses 0, 20, 40, and 60 kGy and a dose rate of 4.98 kGy/h.

**2.2. Characterization of Colloidal AgNPs.** The formation, morphology, and dispersion of colloidal AgNPs were

determined to obtain the optimal dose and stabilizer concentration for the irradiation process. UV-Vis spectrophotometry was used to determine the presence of AgNPs which showed a characteristic absorption peak of  $\text{Ag}^0$  distinctive from the starting  $\text{Ag}^+$ . After the irradiation process, solutions containing colloidal AgNPs at all treatments were diluted by water to 0.16 mM calculated per the starting  $\text{AgNO}_3$  solution and the UV-Vis spectra were recorded by a spectrophotometer (Evolution 300, Thermo Scientific). The morphology and dispersion of AgNPs were examined by transmission electron microscopy (TEM, Hitachi HT7700). The diameters of AgNPs were measured from  $\sim 50$  particles for treatments that yielded the uniform dispersion of particles and were represented as mean  $\pm$  standard deviation.

**2.3. Preparation of SF-CS-PVA Wound Dressing Films.** A 2% (w/v) CS solution was first prepared in 1% (v/v) acetic acid and the pH was adjusted to 6.5. Subsequently, 2% (w/v) SF powder was added to the CS solution and stirred overnight at RT until completely dissolved. The mixed SF-CS solution was concentrated into a paste-like form by rotor evaporation before a 15% (w/v) PVA solution was added into the paste to obtain the final concentration of 20, 33, 50, and 67% (w/w). After being thoroughly mixed by stirring, the paste was cast into  $5 \text{ cm} \times 7 \text{ cm} \times 0.1 \text{ cm}$  Teflon molds and rendered crosslinked by exposure to gamma rays from a Co-60 source (Gems Irradiation Center, Thailand Institute of Nuclear Technology (Public Organization)) for a total dose of 80 kGy at a dose rate of 9.52 kGy/h. For the evaluation of antibacterial properties, AgNPs at varied concentrations were added to the selected paste formulation prior to gamma irradiation.

**2.4. Characterization of SF-CS-PVA Films.** Using universal testing machine (Cometech, B2-type), the mechanical properties including elastic modulus, stress at break, and strain at break of SF-CS-PVA films were determined. Samples were cut into  $1 \text{ cm} \times 4 \text{ cm}$  strips and mounted on 50 N load cell that moved at a speed of 50 mm/min. Data were collected as load and displacement from 3–5 samples per PVA concentration. The elastic modulus was calculated from stress-strain curves.

In addition to the mechanical properties, the ability of films to absorb water was determined by the degree of swelling. Samples were incubated in 1X phosphate buffer saline (1X PBS) containing calcium and phosphate ions at  $37^\circ\text{C}$  for 72 h, blotted with kimwipes paper and weighed. The degree of swelling was calculated according to the following formula [18]:

$$\text{Degree of swelling (\%)} = \frac{W_s - W_d}{W_d} \times 100, \quad (3)$$

where  $W_s$  is the weight of film in its swollen state and  $W_d$  is the film's dry weight. Films with optimal SF-CS-PVA formulation were chosen as the wound dressing model for the evaluation of antibacterial properties.

**2.5. Evaluation of Antibacterial Properties.** The ability of colloidal AgNPs to serve as antiseptic agent was evaluated by disc diffusion assay at varied AgNPs concentrations from 0 to 2.160 ppm. To confirm the presence of AgNPs,

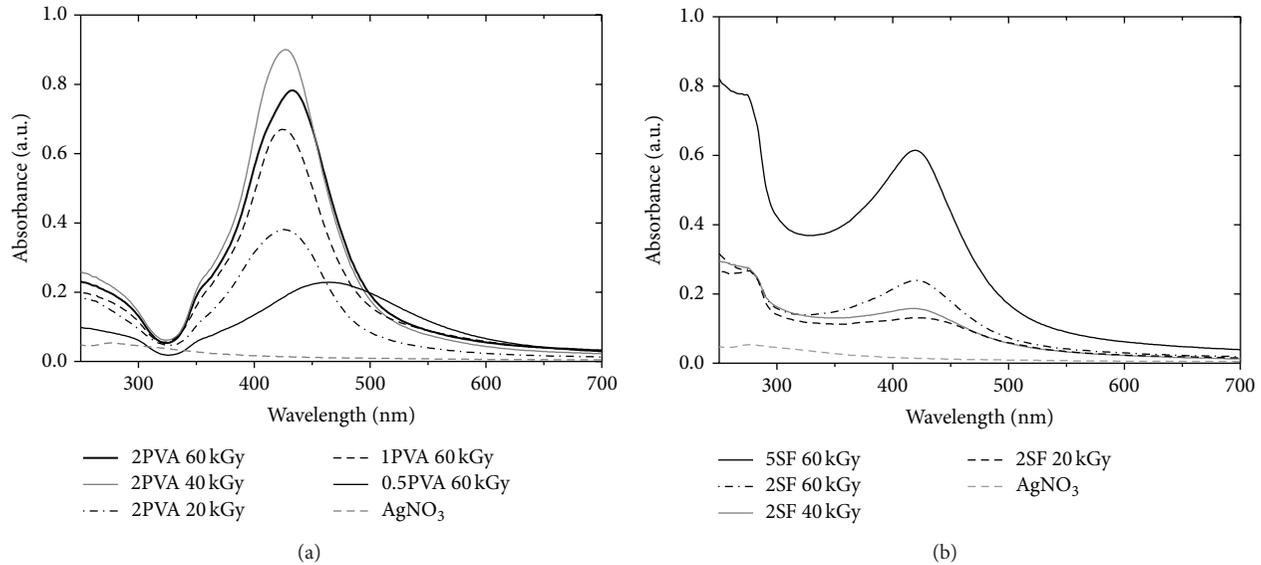


FIGURE 1: UV-Vis spectra of colloidal AgNPs synthesized by gamma radiolysis with (a) PVA or (b) SF as stabilizers at different irradiation doses and stabilizer concentrations.

AgNP-embedded films were inspected by scanning electron microscopy (SEM, JOEL JSM 6380 LV) and the presence of Ag was analyzed by energy dispersive X-ray spectrometer (EDS). Films were cut into circular discs 8 mm in diameter, placed on Mueller-Hinton agar plates that were preinoculated with  $10^6$  colony forming unit (CFU)/mL of either *S. aureus* or *P. aeruginosa* and incubated at  $37^\circ\text{C}$  for 18 h. The clear zones surrounding the discs, which indicated inhibition of bacterial growth, were recorded at the termination of incubation period and the inhibition ratios were calculated as the average diameters of zones of inhibition divided by the disc diameters. Data were represented as mean  $\pm$  standard deviation from 8 replicates.

### 3. Results and Discussion

**3.1. Effect of Irradiation Dose on the Formation of Colloidal AgNPs.** After exposure to gamma irradiation, the formation of colloidal AgNPs in solutions could be observed by changes in color of the starting  $\text{AgNO}_3$  from clear to reddish brown for AgNPs stabilized by PVA and dark brown for AgNPs stabilized by SF. For well dispersed AgNPs with size of a few nanometers, the optical property determined by UV absorption exhibits an intense peak characteristic to  $\text{Ag}^0$  at wavelengths 380–440 nm as reported in previous studies [5, 8, 10–12]. Here we first tested the effect of irradiation dose on the formation of colloidal AgNPs.

At the highest PVA concentration (2PVA, Figure 1), the presence of AgNPs was indicated by UV-Vis spectra which showed the maximum absorption wavelength ( $\lambda_{\text{max}}$ ) at 425–432 nm. As an internal control, the absorption band was absent in the spectra of  $\text{AgNO}_3$ . The highest absorbance intensity was obtained at irradiation dose of 40 kGy with a slight shift towards shorter wavelength. For colloidal AgNPs capped with SF (Figure 1(b)),  $\lambda_{\text{max}}$  at 420 nm was distinct when the irradiation dose was raised to 60 kGy, whereas

TABLE 1: Effect of irradiation dose and stabilizer concentration on the UV absorption of colloidal AgNPs.

Stabilizer (% w/v)	Dose (kGy)	$\lambda_{\text{max}}$ (nm)	Absorbance (a.u.)
0.5PVA	20	447	0.177
0.5PVA	40	432	0.316
0.5PVA	60	463	0.229
1PVA	20	424	0.307
1PVA	40	419	0.451
1PVA	60	421	0.57
2PVA	20	425	0.381
2PVA	40	427	0.901
2PVA	60	432	0.783
2SF	20	415	0.131
2SF	40	414	0.158
2SF	60	419	0.239
5SF	20	421	0.206
5SF	40	419	0.4
5SF	60	419	0.615

Note: UV-Vis spectra could not be observed for AgNPs prepared in SF solutions at concentrations below 1% (w/v).

it almost diminished at lower doses. Table 1 summarizes UV absorption data of AgNPs at all irradiation doses and stabilizer concentrations tested in this present study.

The morphology of AgNPs was further examined by TEM. For AgNPs capped with 2% (w/v) PVA, large aggregates surrounded by small particles (diameter  $\sim 28$  nm) formed at irradiation dose of 20 kGy, whereas a uniform dispersion of individual, small particles with diameters  $32.3 \pm 4.40$  and  $41.1 \pm 4.00$  nm was obtained at doses 40 and 60 kGy, respectively. The slight increase in particle size at higher irradiation dose may be due to degradation of PVA, which could be less effective in preventing the growth of particles.

TABLE 2: The mechanical properties and degree of swelling of SF-CS-PVA films at varied PVA concentration. Data are represented as mean  $\pm$  standard deviation.

% PVA (w/w)	Stress at break (kPa)	Strain at break (%)	Elastic modulus (kPa)	Degree of swelling (%)
20	109.6 $\pm$ 40.3	18.0 $\pm$ 3.4	1111.6 $\pm$ 415.9	134.15 $\pm$ 2.72
33	313.8 $\pm$ 35.0	27.8 $\pm$ 2.4	1960.5 $\pm$ 160.2	131.37 $\pm$ 1.98
50	346.2 $\pm$ 39.9	36.8 $\pm$ 4.6	2567.3 $\pm$ 288.8	148.80 $\pm$ 3.11
67	374.1 $\pm$ 28.82	68.9 $\pm$ 4.3	2242.7 $\pm$ 493.5	166.27 $\pm$ 2.33

This observation is similar to the previous report of chitosan-capped AgNPs prepared at high irradiation dose [10]. For AgNPs capped with SF, large aggregates as well as small particles were observed at the irradiation dose of 60 kGy. Interestingly, these small particles (diameter  $\sim$  40 nm, Figure 2(d)) remained connected and were not fully dispersed. This can be attributed to the less hydrated nature of SF protein compared to PVA that makes it less effective in shielding individual particles apart from each other. Based on these data, the extent of irradiation dose in our experimental conditions plays a major role in the formation of small Ag<sup>0</sup> particles.

**3.2. Effect of Stabilizer Concentration on the Formation of Colloidal AgNPs.** Agglomeration of Ag<sup>0</sup> into large particles can be prevented by stabilizers that envelop over the particle surface. In this report, PVA and SF at varied concentrations were tested to optimize the morphology and dispersion of AgNPs. At the same irradiation dose of 60 kGy, the UV-Vis spectra showed narrower absorption bands with shifts towards shorter wavelength and higher peak intensity as the PVA concentrations increased from 0.5 to 2% (w/v) (Figure 1(a)). These changes may correspond to the formation of smaller particles with narrow distribution in particle size [10] as the red shifts and broadening of the band are due to agglomeration of Ag<sup>0</sup> into large clusters [5]. Similarly for AgNPs capped with SF, the absorption band with strongest peak intensity corresponded to AgNPs prepared in 5% (w/v) SF solution (Figure 1(b)), whereas AgNPs prepared with lower SF concentrations contributed to absorption bands with lower intensities.

In line with UV-Vis spectra, the majority of particles were in large aggregates (Figure 2(e)) at the lowest PVA concentration, whereas a mix of large aggregates and small particles was observed at 1.0% (w/v) PVA (Figure 2(f)) and the uniform dispersion of AgNPs was obtained at 2% (w/v) PVA (Figures 2(b) and 2(c)). Thus, a sufficient concentration of PVA was required to prevent particle agglomeration. Taken all together, both the irradiation dose and PVA concentration affect the formation and dispersion of Ag<sup>0</sup> into small, individual particles in our study. Based on the morphology and uniform dispersion of colloidal AgNPs, we selected AgNPs prepared with 2% (w/v) PVA at irradiation dose of 40 kGy to be incorporated into dressing films for the evaluation of antibacterial properties.

**3.3. Characterization of SF-CS-PVA Films.** Using radiation processing technique, polymeric films can be crosslinked and

simultaneously sterilized in a single step [19], which is advantageous for the fabrication of biomedical products. In this report, we also utilized gamma radiation to process wound dressing films composed of SF, CS, and PVA. The optimal formulation of SF-CS-PVA films was chosen based on the films' mechanical and swelling properties as summarized in Table 2. In terms of the mechanical properties, the stress at break, strain at break and elastic modulus were shown to increase with the PVA concentration. As the proportion of PVA was raised to more than 50% (w/w), the elastic modulus and stress at break did not change significantly although the strain at break continued to increase with PVA concentration.

The ability to absorb fluid is one of the key aspects of wound dressing, which can be characterized by the degree of swelling. Our data showed that the degrees of swelling of SF-CS-PVA films at all PVA concentrations were more than 100% with a slight increase with PVA concentration. These values were in agreement with other PVA-based hydrogels in previous report [20]. Based on these data, the formulation of the film was selected to comprise 50% PVA (w/w), which possessed the highest content of natural polymer components while still maintaining the optimal mechanical and swelling properties.

**3.4. Inhibition of Bacterial Growth.** The SF-CS-PVA films incorporated with AgNPs at concentrations 0–2.160 ppm were evaluated for antibacterial properties against *S. aureus* and *P. aeruginosa* by disc diffusion assay. The distribution of AgNPs throughout the film was confirmed by EDS signals taken over the cross-sectional area of the film (Figure 3). Prominent clear areas (Figure 4), indicating the zones of growth inhibition, around the films on plates inoculated with either bacterial strain were observed when AgNP concentration was raised  $\geq$ 432 ppm and increased with the amount of AgNPs in the films. As an internal control, no clear zone was observed around films without AgNPs.

For *S. aureus*, the inhibition ratios, calculated as the average diameters of zones of inhibition divided by the disc diameters, increased from 1.46  $\pm$  0.14 at 432 ppm AgNPs to 1.67  $\pm$  0.18 at 2160 ppm AgNPs. Similarly, the inhibition ratios for *P. aeruginosa* were 1.44  $\pm$  0.16 and 1.59  $\pm$  0.19 at 432 ppm and 2160 ppm AgNPs, respectively. In our study, the minimum concentration of AgNPs in the films that exhibited antibacterial properties against both strains of bacteria was less than the amount of Ag compounds used in some commercial wound dressings [7]. Therefore, we have demonstrated that colloidal AgNPs can serve as an effective

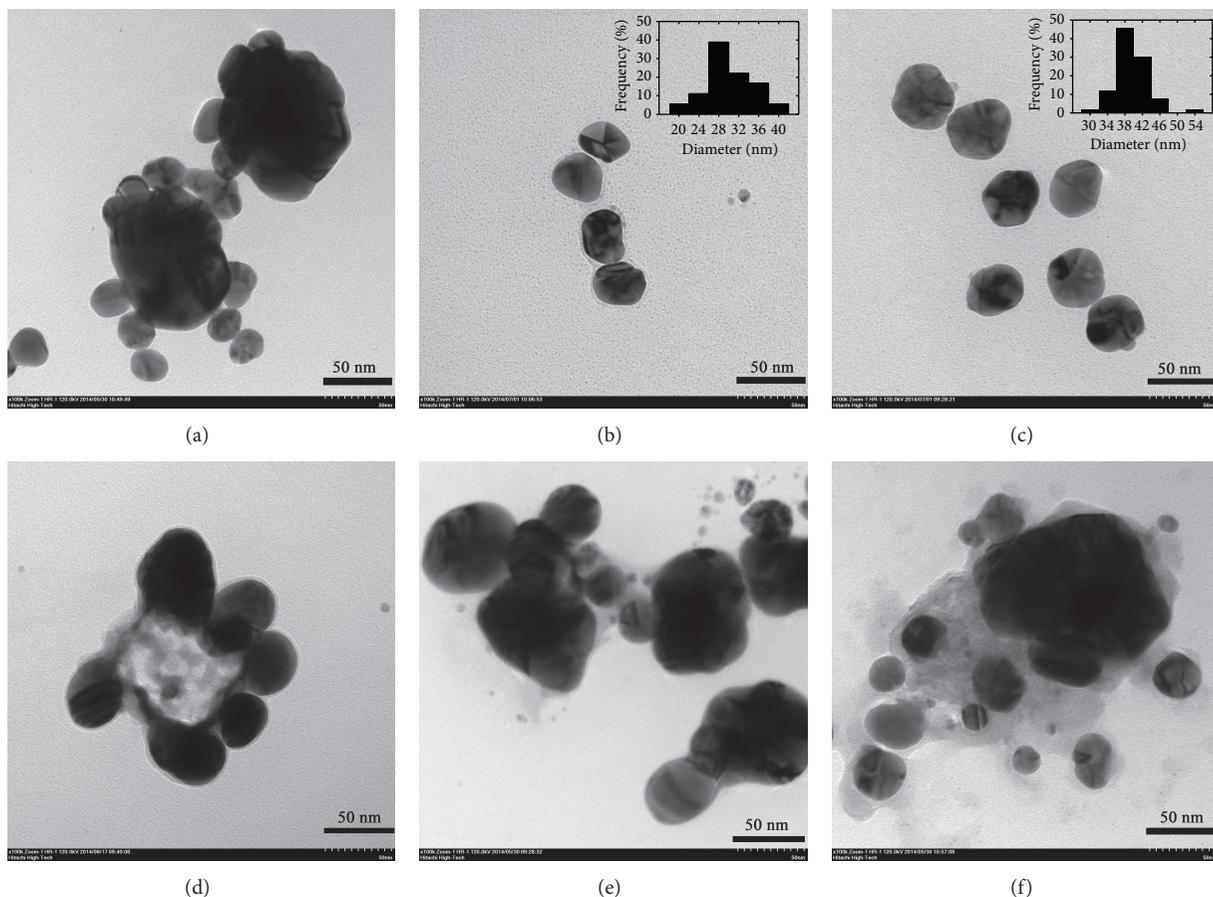


FIGURE 2: TEM images representing the morphology and dispersion of colloidal AgNPs. Top panel are images of AgNPs prepared in 2% (w/v) PVA solutions at irradiation doses of (a) 20, (b) 40, and (c) 60 kGy. Insets show histograms of size distribution. In lower panels, AgNPs were formed at the irradiation dose of 60 kGy in (d) 5% (w/v) SF, (e) 0.5% (w/v) PVA, and (f) 1.0% (w/v) PVA solutions.

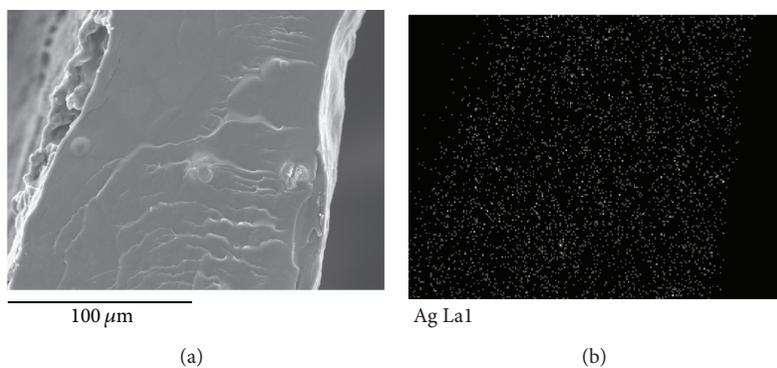


FIGURE 3: Representative SEM images show (a) the cross-section of SF-CS-PVA film incorporated with AgNPs at a concentration of 2,160 ppm and (b) collected EDS signal of silver over the cross-sectional area.

antiseptic agent when incorporated into films, which can potentially be used in wound healing application.

#### 4. Conclusion

Gamma radiation was applied in the radiolytic synthesis of colloidal AgNPs and processing of films for wound dressing

application. The size and dispersion of particles were shown to depend on the irradiation dose and concentration of stabilizer in which the uniform dispersion of AgNPs with size in the range of 30–40 nm was achieved at doses  $\geq 40$  kGy and 2% (w/v) PVA. When incorporated into SF-CS-PVA films, the concentration of AgNPs at 432 ppm was found to be effective in inhibiting the growth of bacteria.

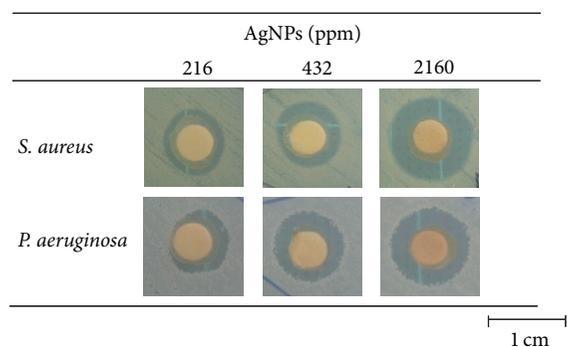


FIGURE 4: Disc diffusion assay shows the antibacterial properties of SF-CS-PVA films embedded with AgNPs. The inhibition of bacterial growth appears as clear zones surrounding the films.

### Conflict of Interests

The authors declare that there is no conflict of interests regarding the publication of this paper.

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## Review Article

# Preparation of Silver Nanoparticles and Their Industrial and Biomedical Applications: A Comprehensive Review

**Adnan Haider and Inn-Kyu Kang**

*Department of Polymer Science and Engineering, Kyungpook National University, Daegu 702-701, Republic of Korea*

Correspondence should be addressed to Inn-Kyu Kang; [ikkang@knu.ac.kr](mailto:ikkang@knu.ac.kr)

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Silver nanoparticles (Ag-NPs) have diverted the attention of the scientific community and industrialist itself due to their wide range of applications in industry for the preparation of consumer products and highly accepted application in biomedical fields (especially their efficacy against microbes, anti-inflammatory effects, and wound healing ability). The governing factor for their potent efficacy against microbes is considered to be the various mechanisms enabling it to prevent microbial proliferation and their infections. Furthermore a number of new techniques have been developed to synthesize Ag-NPs with controlled size and geometry. In this review, various synthetic routes adapted for the preparation of the Ag-NPs, the mechanisms involved in its antimicrobial activity, its importance/application in commercial as well as biomedical fields, and possible application in future have been discussed in detail.

## 1. Introduction

Nanotechnology is booming by leaps and bounds due to the emergence of nanomaterials (particularly inorganic nanoparticles (NPs) and nanorods) with unique functions and size dependent physicochemical properties differing significantly from their bulk counterpart [1]. The potential of inorganic NPs has been explored worldwide in nanomedicine, drug delivery and biomedical devices, cosmetics, electronics, energy sector, and environmental protection [2–4]. Among inorganic NPs, silver nanoparticles (Ag-NPs or nanosilver), due to its novel chemical, physical, and biological properties as compared to their bulk form, have attracted the attention of researchers from various academic laboratories [5]. Ag-NPs have distinctive physical and chemical properties, for example, high thermal and electrical conductivity, surface-enhanced Raman scattering, chemical stability, catalytic activity, and nonlinear optical behavior [6]. These properties take Ag-NPs to the top of the priority list, to be used in inks, in electronics, and for medical purpose [7].

Furthermore, Ag-NPs are widely known for its antimicrobial properties against microbes such as bacteria, fungi, and virus [8]. Due to their proven antimicrobial properties, Ag-NPs are widely used in the daily used commercial products,

such as plastics, food packaging, soaps, pastes, food, and textiles, which has increased their market value to a great extent [9]. Its importance can be judged from the fact that Ag-NPs can be used in various form, such as colloidal (enamel, coating, and in paints), in liquid form (shampoo), or in solid form (blending Ag-NPs with a solid material such as polymer scaffolds) and even can be found suspended in materials like soap and nonwoven fabrics. Ag-NPs importance cannot be neglected even in textile industry, where Ag-NPs are used in the water filtration membranes. The idea behind the use of Ag-NPs in the water filtration membrane is based on the utilization of their proven antimicrobial properties and slow release rate of Ag-NPs from the membrane. The slow release rate prolongs capability of the membrane to be used as a protective barrier against various bacterial and other pathogenic microbes present in the water [9, 10].

Recently, the number of publications on the topic of Ag-NPs has increased rapidly and an increase of 93% in the number of published articles has been observed since 2001–2011. During this period, 247 articles were published in 2001, which in 2011 increased to 3603 articles. Most of these were published in the area of chemistry, materials science [10], physics, engineering, polymer science, spectroscopy, electrochemistry, molecular biochemistry, optics, and spectroscopy.

A large number of consumer products having commercial value are being prepared parallel, banking on the literature published in the above mentioned period in the area of synthesis and applications [10].

Since vast literature on the Ag-NPs is present in the form of individual research articles that only focuses on the minor application part, we aimed to summarize it in the present review form so that the interested readers may find all recent information ranging from synthetic procedures to application in a single document. This paper aims to summarize the various methods adapted for the synthesis of Ag-NPs and the area in which these Ag-NPs were, are, and to be used so that it can be easy for common readers who wants to seek information related to these nanoparticles (see Figure 1). In this review paper every aspect of Ag-NPs, from synthesis to their application in the fields of environment, science, biology, molecular science, textiles industry, and last but not the least their role in medicinal fields, has been briefly explored. Besides, the future prospect of these nanoparticles in day to day life was also discussed.

## 2. Synthetic Routes Adapted for the Synthesis of Ag-NPs

Currently, variety of methods, such chemical, physical, photochemical, and biological, has been employed for the synthesis of Ag-NPs as depicted in Figure 2. Each method has its merits and demerits. The synthetic routes with commonly associated problems, that is, costs, stability, scalability, particle sizes, and size distribution for the Ag-NPs, have been described as follows.

**2.1. Chemical Method.** Among the existed reported methods, so far, chemical methods are preferred for the preparation of Ag-NPs due to the ease in synthesizing them in solution [11]. Many research groups and academia are using these methods to synthesize Ag-NPs in various sizes and shapes. For example, one research group synthesized monodisperse silver nanocubes by simply reducing  $\text{Ag}(\text{NO}_3)_3$  with ethylene glycol in the presence of polyvinylpyrrolidone (PVP) polymer [12]; the process was called polyol process. In this process, it has been revealed that ethylene glycol works as both the solvent and the reducing agent. Furthermore, the size and shape of the nanocubes were dependent on the molar ratio of  $\text{Ag}(\text{NO}_3)_3$  and PVP. Thus by controlling the experimental parameters, the geometry (size and shapes) of the Ag-NPs can be tailored. Round shaped Ag-NPs with a controlled size and monodispersity were synthesized by modifying the polyol method using precursor injection. In this method, particles with 20 nm or smaller size were prepared. The governing factors of the precursor injection method were precursor injection rate and in situ conditions (inside the reaction mixture). The injection precursor method proved to be effective in synthesizing particles with brilliant control on the size for the monodispersion [13]. In another reported method, monodispersed Ag-NPs were synthesized using  $\text{Ag}(\text{NO}_3)_3$ , oleylamine, and liquid paraffin. Oleylamine-paraffin system was used for controlling high temperature which paid pivotal role in determining the particle size

of the synthesized Ag-NPs. Liquid paraffin was used to sustained high temperature and secondly helped in avoiding the use of solvents that would hamper the whole synthetic process [14]. Normally, the synthesis of Ag-NPs by chemical method banks on three factors (stages): (a) Ag precursor, (b) reducing agents, and (c) stabilizing agent. Furthermore, the synthesis and geometry of Ag-NPs rely on the nucleation and subsequent stacking of the Ag nuclei. Uniform size and monodispersity can be achieved by controlling the nucleation stage and stacking of nuclei in which intern depends on experimental parameters such as precursor, pH, temperature, and reducing agents. More recently, Mukherji and Agnihotri synthesized Ag-NPs using  $\text{Ag}(\text{NO}_3)_3$  as precursor and sodium borohydride and trisodium citrate as reducing and stabilizing agent. The size of the Ag-NPs was controlled by optimizing the experimental parameters and was in the range of 5 nm to 100 nm. Furthermore, they elucidated the effect of size and dose dependent property of the synthesized Ag-NPs. From the analysis of the obtained results, they revealed that Ag-NPs with small size show excellent antibacterial activity as compared to its other counterparts [15].

The formation of colloidal solutions from the reduction of silver salts involves two stages of nucleation and subsequent growth. The authors also revealed that the size and the shape of synthesized Ag-NPs are strongly dependent on aforementioned stages. Furthermore, for the synthesis of monodispersed Ag-NPs with uniform size and monodispersity, simultaneous formation of nuclei is required. By doing so all of the nuclei are likely to have the same or similar size, which will be followed by subsequent growth. The initial nucleation and the subsequent growth of nuclei can be controlled by adjusting the reaction parameters such as reaction temperature, pH, precursors, reduction agents (i.e.,  $\text{NaBH}_4$ , ethylene glycol, and glucose), and stabilizing agents (i.e., PVA, PVP, and sodium oleate) [16, 17].

**2.2. Physical Method.** Along with the chemical methods used, various alternative techniques were also adopted by researchers for the synthesis of Ag-NPs. Usually in those techniques, evaporation and condensation processes are implemented for the synthesis of Ag-NPs. Similar to chemical method, these methods have their own merits and demerits. One of the most common drawbacks of these methods is the higher energy requirement and time consuming. Therefore, researchers have reported numerous alternative physical methods for the synthesis of Ag-NPs instead of implementing conventional condensation and evaporation method. These methods not only reduced the preparation time but also are energy friendly. For example thermal-decomposition method was implemented for the synthesis of Ag-NPs in solid form. In such method, complexation reaction between Ag and oleate at elevated temperature was carried out for the synthesis of Ag-NPs with particle size less than 10 nm [22]. In another work Jung et al. reported that Ag-NPs with uniform size and monodispersion can be prepared using ceramic heating system, for supplying continuous heat without any fluctuation [23]. The heating system was used in order to evaporate the precursor used in the preparation of Ag-NPs. Tien et al. used arc discharge technique for the synthesis

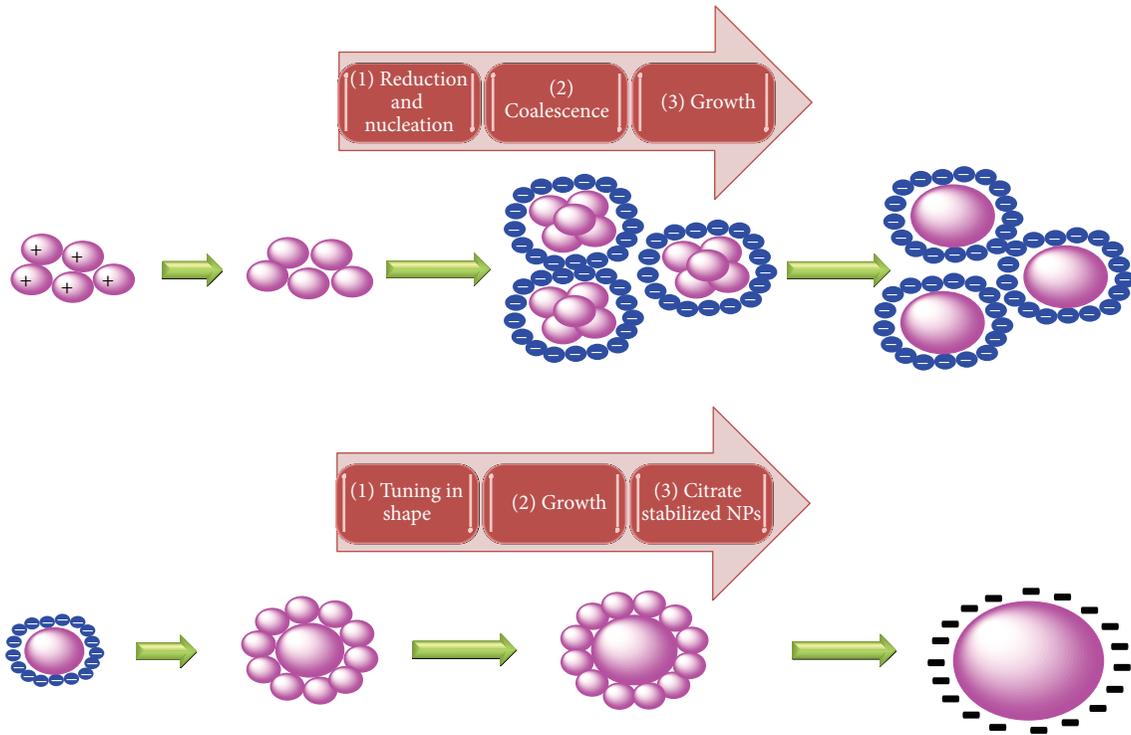


FIGURE 1: Schematic representation of size controlled synthesis of Ag-NPs using coreduction method.

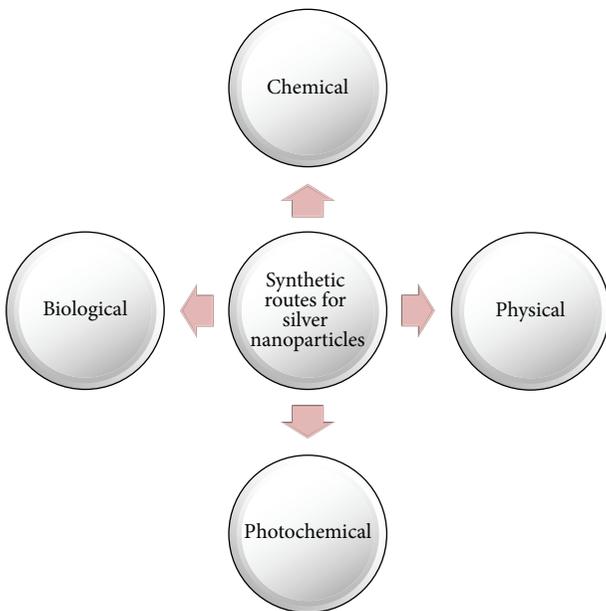


FIGURE 2: Various synthetic routes adopted for the synthesis of Ag-NPs with uniform geometry.

of Ag-NPs in DI water, avoiding the use of surfactant. The authors used electrodes made of silver that were dipped in DI H<sub>2</sub>O. The results reported by Tien et al. using arch discharge technique showed that the size of the synthesized Ag-NPs was less than 10 nm [24]. Later on Kuo et al. used electric discharge machining system for the synthesis

of Ag-NPs by optimizing the experimental parameters. The authors performed experiment for elucidating the effect of the process parameters on the characteristic of Ag-NPs [10, 25, 26]. Recently researchers reported a unique technique for the physical synthesis of Ag-NPs with uniform geometry and particle size less than 5 nm along with improved dispersion. The technique was proposed to be an alternative for chemical techniques with the added advantage of saving time. Ag-NPs were also prepared by sputtering of metal into reaction mixture, that is, physical deposition of Ag into glycerol. Physical techniques used for the preparation of Ag-NPs having uniform particle size and shape are mostly govern by the thermal, ac power, and arc discharge. By adopting these aforementioned techniques, bulk amount of Ag-NPs can be synthesized in a single process which will save time and the purity of the Ag-NPs particles will not be compromised. However, the cost is considered to be the main hurdle in adopting such method as it needs expensive equipment [10].

2.3. *Photochemical Synthesis.* In the photochemical techniques mainly two different methodologies are implemented for the synthesis of Ag-NPs: (a) photophysical and (b) photochemical techniques. In the photochemical techniques Ag-NPs are synthesized by photoreduction of precursor or Ag ions using photochemically activated intermediates such as radical [27]. In one of the method Ag-NPs were synthesized using UV radiation and aqueous solution containing Triton x-100 which acts as stabilizing agent. The surfactant used in the study helped in maintaining the stability, monodispersion, and uniform size of the synthesized Ag-NPs [28]. In another reported method, Ag-NPs were synthesized

from aqueous solution of alkali containing  $\text{AgNO}_3$  and carboxymethylated chitosan (CMCTS) using UV radiation. CMCTS was used as reducing agent with the added advantage of enhancing the stability of the synthesized Ag-NPs. The diameter of the synthesized Ag-NPs was less than 10 nm. The stability of synthesized Ag-NPs was more than 6 months in the alkali/CMCTS alkali solution [29]. Balan et al. reported an improved photochemical method for the synthesis of Ag-NPs which involved the direct photoreduction of  $\text{AgNO}_3$  with the help of laser source capable of emitting radiation in the range of near infrared (IR) region. They used dyes as photoactive agents [30]. The merits of photochemical techniques for the preparation of Ag-NPs were (a) high purity of the synthesized Ag-NPs and easy processing; (b) Ag-NPs were prepared by UV-radiation with the help of reducing agents; (c) wide range of reaction medium can be used that can be glass, polymer, micelles, emulsion, and so forth [31].

**2.4. Biological Synthesis.** Using conventional methods for the synthesis of Ag-NPs requires (a) Ag precursors, (b) reducing agent, and (c) stabilizer/capping agent (PVP) (for avoiding agglomeration of the newly synthesized Ag-NPs). However in biological techniques, biomolecules replaced the conventional reducing and stabilizing agents. In biological methods Ag-NPs are synthesized using plants (such as algae, yeast, fungi, and bacteria) as reducing and stabilizing agents [32]. *Shewanella oneidensis* (a metal reducing agent) was used for the biosynthesis of Ag-NPs using  $\text{Ag}(\text{NO}_3)$  solution as precursor. The synthesized Ag-NPs size was less than 15 nm, with uniform dispersion, spherical shape, enhanced stability, and large surface area. Such methods used for the synthesis of Ag-NPs are highly economical and reproducible and consume lesser energy contrary to the conventional methods [33]. In another study, *Trichoderma viride* fungus was used for the biosynthesis of Ag-NPs from  $\text{Ag}(\text{NO}_3)$  precursor [34]. The geometry of the biosynthesized Ag-NPs was highly variable with particles size less than 50 nm. Furthermore, stable Ag-NPs with size less than 20 nm were synthesized by using airborne bacteria (*Bacillus* sp.) using  $\text{Ag}(\text{NO}_3)$  as the precursor. The biosynthesized Ag-NPs were collected from the periplasmic region (a space between outer and inner membrane) of the bacterial cell. Moreover, spherical Ag-NPs synthesis was reported by the reduction of  $\text{Ag}(\text{NO}_3)$  phyllanthin extract at room temperature. The size and shape of the Ag-NPs were governed by concentration of phyllanthin extract [35, 36]. In another study published by Venkata Subbaiah and Savithamma, the authors revealed that Ag-NPs can also be synthesized from *Cadaba Fruticosa* leaves using  $\text{Ag}(\text{NO}_3)$  as precursor. The biological synthesized nanoparticles were very potent against microbes [37].

### 3. Effect of Shape, Size, and Chemical Forms of Ag-NPs

Many scientists have reported on the particle size dependent activity of Ag-NPs, although it is not a rule [38, 39]. Powers et al. presented size dependent properties of PVP-capped Ag-NPs. In others studies, researchers suggested that the smaller is the size the more is the cytotoxicity. This could be attributed

to the fact that small sized Ag-NPs have easier uptake, easy dissolution, and smooth release of Ag-NPs ions along with increased surface area [40]. Some of the studies emphasized on the mass based property of Ag-NPs without taking particle size and surface area into consideration. It has also been proposed that surface modification of Ag-NPs enhances the dissolution and stability of these nanoparticles (for longer time) [38, 39]. As biomolecules, coating and capping agents can affect the size, shape, and interfacial properties of the Ag-NPs.

Therefore researchers have highlighted the fact that size shape and surface area are the governing factor in determining the extent of cytotoxicity of Ag-NPs. For this reason numerous techniques such as dynamic light scattering (DLS), transmission electron microscope (TEM; see Figure 3), and centrifugation have been employed to facilitate accurate measuring of size and dispersion of these nanoparticles [41, 42].

### 4. Application of Ag-NPs

Ag-NPs have been in use for more than 150 years and are recognized as antimicrobial agent in United States (USA) since 1954 [43]. There are many assumptions about the use of silver by the ancient Egyptian and Romans. The most stable oxidation state of Ag is +0 and +1 although it can exist in other oxidation states as well and can form various complexes.  $\text{Ag}(\text{NO}_3)$  is considered as a precursor for the synthesis of Ag-NPs. The size and geometry of Ag-NPs are dependent on the synthetic route adopted for its synthesis; however it can be found in spherical, rod, and triangular shape, or coated with polymer, biomolecules, and sugars. Ag-NPs has numerous chemical, physical and biological functions which are explained point by point.

**4.1. Textiles.** Fabrication of functionalized fabrics with Ag-NPs has a fair share in the functionalized fabricated materials [44]. Blaser et al. proposed an exposure model in which fabrics functionalized with Ag-NPs and polyethylene bags are considered to be the prime governing factors for presence of silver in the atmosphere [45]. Garments such as socks, T-shirts, sports-wear are functionalized with Ag-NPs, but the most advantageous use of Ag-NPs is considered to be in medical field because of the high risks of contamination associated with surgical suits [46]. Freeman et al. reported the effect of Ag-NPs functionalized fabrics on bacterial contamination.

Freeman et al. reported the effect of Ag-NPs functionalized fabrics on bacterial contamination. Which stated that Ag-NPs functionalized fabricated fabric has a prime role in retarding the bacterial colonial growth [10, 32, 47–49]. Various techniques are adopted for the fabrication of fabric functionalized with Ag-NPs; for example, the most famous and talked about techniques used for the fabrication of silver containing fabrics are by blending Ag-NPs with the fabricated material so that the Ag-NPs are embedded inside the functionalized fabrics or secondly by the surface immobilization of fabrics (to be functionalized) with Ag-NPs. However, along with the merits such fabrics do have demerits such as release of Ag ions during washing which is considered to be the prime concern regarding the durability of such Ag-NPs functionalized materials [47, 48, 50]. Furthermore

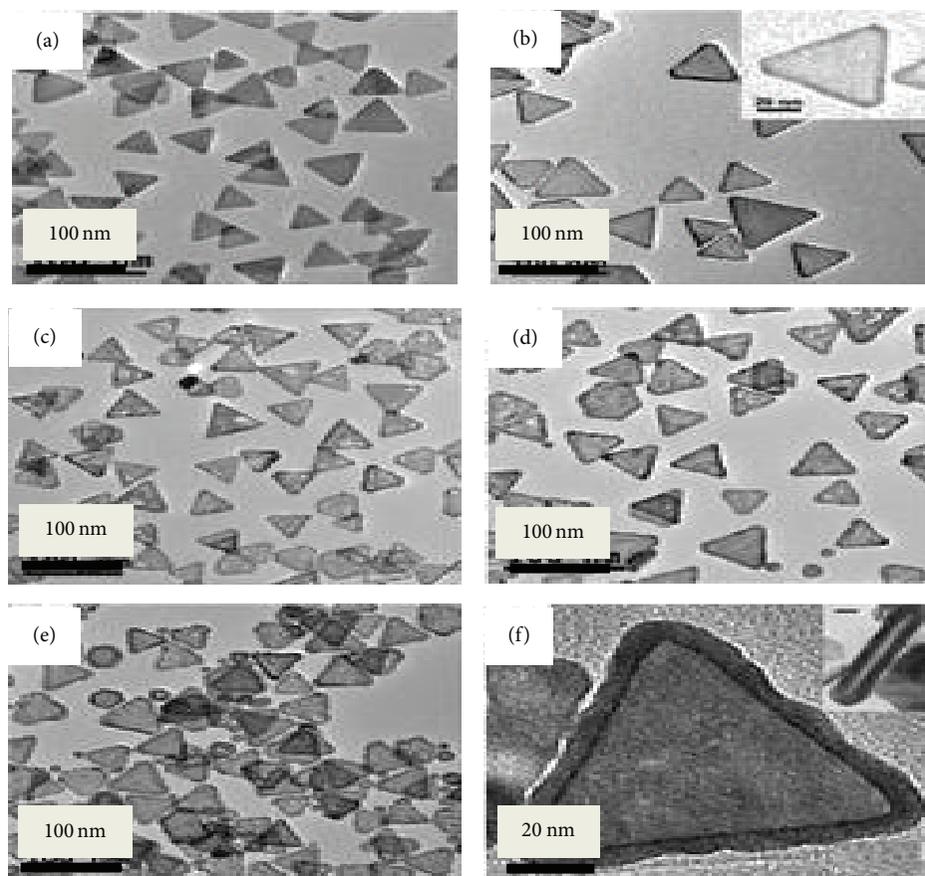


FIGURE 3: TEM images of (a) initial silver nanoprisms; (b) samples at 45 min with gold deposition on the prism edges; (c) samples from stage 2 with some small pinholes; (d) samples with partially refilled pinholes after 120 min (stage 3); (e) samples at 200 min with full gold shells (stage 4); (f) a typical final gold-coated nanoprism (the inset is the cross-sectional view with a scale bar of 10 nm). Reproduce from Shahjamali et al. with permission from Wiley publisher [18].

composite fabrics containing Ag-NPs present in the sheath part revealed promising antibacterial activity on the contrary to the fabrics containing Ag-NPs in the core part [51].

**4.2. Food Packaging.** As already discussed the merits of Ag-NPs functionalized fabrics likewise Ag-NPs have also made its mark in the food packaging industry and are known to be one of the important components in the packaging materials used for preservation of food for longer duration. For example, materials whose surfaces are coated with Ag-NPs can be useful in preventing the preserved food from contamination (preventing contamination caused by microbes) due to slow release of Ag-NPs from the coated surface along with preventing growth of the microbes on the surface of the packaging material (effect of Ag-NPs against microbes will be discussed in detail in the later part of this review). Researchers working in the field of food preservation have reported sonochemical coating, a simple and versatile technique used for preparation of coating materials from the colloidal solution containing Ag-NPs using ultrasonication [52]. The coating proved to be effective against various strains of bacteria (gram-negative *E. coli* and gram-positive *S. aureus* bacteria). The method proved to be a step forward towards

the synthesis of materials with the help of which food can be preserved for longer duration. Cushen reported that Ag-NPs have significance in research relating food preservation and packaging industry. However only limited forms of Ag-NPs are allowed by the EFSA (European Food Safety Authority) which can be used in food packaging and preservation [53].

**4.3. Plastic Coatings.** The importance of Ag-NPs in preparation of medical devices can be judged from the fact that a wide range of medical devices are prepared from it due to its potency against various strains of bacteria (gram positive and gram negative). Catheters are usually prepared from materials that contain Ag-NPs in order to avoid infection and contamination. These catheters are useful in sustain and targeted release of Ag ions from the Ag-NPs which ultimately prevents microbial activity. Roe et al. synthesized catheters coated with Ag-NPs. From the obtained results the authors concluded that along with inhibiting the formation of biofilm these catheters were nontoxic and showed sustained release of Ag-NPs [54]. In hospitals products made from Ag-NPs are used as protecting materials against microbial activity; usually these protecting materials are made up of plastic containing Ag-NPs on the surface or inside the plastic

materials. Furthermore materials coated with Ag-NPs can be used in preventing microbial activity in water; for example, Doolette et al. revealed that Ag-NPs coating can be effective in preventing the microbial activity in water [55, 56].

**4.4. Nanoprism Preparation.** Recently scientist has diverted their attentions towards the synthesis and optical activity of Ag-NPs nanoprism. Ag-NPs having unique optical activity which results in surface-plasmon resonance (SPR) peaks at relatively long wavelengths. Focusing on silver, various physical and chemical processes have been adopted for the fabrication of nanoprism. One of the processes for making Ag-NPs nanoprism is lithographic technique (nanosphere lithography (NSL)). In this technique Ag-NPs are synthesized and are kept on solid substrates that are capable of producing nanoprism with control over its geometry. Although these lithography techniques are considered to be an alternative to solution-phase technique, chemical techniques are more versatile compared to physical technique. So far two main chemical techniques are identified as stand-out among all of the other techniques: (a) chemical reduction method and photoinduced aggregation of Ag-NPs [57]. The latter became very popular in recent time. Callegari et al. reported that filters during illumination have direct influence on the morphology of the nanoprism prepared from Ag-NPs. In a detailed mechanistic study, Callegari et al. [58] proposed that the whole process is controlled by charge distribution on the Ag-NPs during photoinduced illumination using a laser beam of specified wavelength. In one of the other studies reported researcher in that group proposed that modifying the photoinduced technique using low-intensity light-emitting diodes (LEDs) with different emission wavelengths in combination with various color filters for illumination allows the preparation of Ag-NPs nanoprism having high aspect ratio, so that the in-plane dipole plasmon resonance is shifted to wavelengths above  $\mu\text{m}$ . Furthermore they also suggested mechanism for justifying their reported work [57, 59]. Silver nanoparticles (Ag-NPs) having large absorption coefficients around  $1.55 \mu\text{m}$  could be used, for coating communication fibers, so that it enables reducing the loss of communication and signal contamination. Moreover it can be used in other fields such as biotechnology, as targeted localized heating system using low energy irradiation [60]. Shah-jamali et al. synthesized surfactant free gold-coated Ag-NPs nanoprism. The gold coating provided stability to Ag-NPs nanoprism against etching, the nanoprism had clean surfaces, and the purity endows those gold coated Ag-NPs nanoprism to be used in fields of biosensing and bioimaging [18].

## 5. Role of Ag-NPs in Environment

Silver nanoparticles (Ag-NPs) are world widely famous for their versatility which they exhibit in terms of applications such as electrical, thermal, and antimicrobial properties. In the past people used Ag for ornamental, medicinal, crockery, clothing, building materials, and coins making and as disinfectant. Reports suggested that Ag-NPs have no side effect on the body and act as antimicrobial agent when used with proper care (proper amount) [61–64]. Previously

silver salts were used for the treatment of mental disorders, drug addiction, and several infectious diseases caused by pathogens [64, 65].

**5.1. Water.** Ag-NPs are widely known for its potent properties against pathogens, preventing infections and antimicrobial activity. With durability and their antimicrobial activity, Ag-NPs are considered to be a useful tool against infectious diseases caused by the microbes present in the environment. Therefore its high potency against various infectious microbes can help in avoiding and curing microbial infections. Recently researchers reported disinfectant ability of colloidal Ag-NPs for the treatment of gastrointestinal bacterial infections. One of the other reported studies proposed that core shell magnet nanoparticles comprised of Ag-NPs are effective disinfectant in water purification system [66]. These core shell magnetic nanoparticles exhibited excellent antimicrobial and antipathogenic properties [67]. Most importantly these core/shell magnets can be successfully recovered from the system with the help of external magnet field. Thus the environment can be protected from the hazardous threat caused by pathogens and from the side effect of core/shell magnetic nanoparticles and can be reused [68]. Furthermore cores/shell magnetic nanoparticle can be used in biomedical application for targeted drug delivery, in disposable wipes, for cleansing hands, in sprays, and in maintaining personal hygiene. In a published article, the authors revealed that the use of filter containing Ag-NPs can ensure 100% bacterial inhibition compared to their counterpart [69].

**5.2. Air.** There is concern over the impact of Ag-NPs to mankind and animals, as several reported studies revealed that Ag-NPs have great penetration ability in different environment. Humans and animals are exposed to these Ag-NPs via breathing, ingestion, through skin, and so forth. Therefore concerns arise regarding the impact of Ag-NPs to human and animals health. Due to the small size these nanoparticles can easily penetrate through different organs and ultimately reach the cytoplasm of the cell. For the confirmation of the cytotoxicity of Ag-NPs through breathing Ji et al. conducted experiments on Sprague-Dawley rats for 4 weeks. With different exposure time to the Ag-NPs the rats did not exhibit any abnormal behavior and no change in-terms of weight was observed because of inhalation of Ag-NPs. Furthermore no remarkable changes in the blood biochemical and haematology were observed [70]. However some scientists have reported that lungs are the primary target which are affected by prolong exposure to Ag-NPs [71]. In one of the other articles published by Lee et al. they have reported that Ag-NPs exposure modulated the expression of several genes associated with motor neuron disorders, neural disease, and immunity system of the body, suggesting the potential toxicity of the Ag-NPs to the neural and immune system of the body [72]. Minor pulmonary cytotoxicity (inflammation) in mice was observed after exposing them for longer duration to Ag-NPs [73]. Kim et al. investigate the oral toxicity of Ag-NPs for 4 weeks in Sprague-Dawley rats. It was observed from the experiments that changes in weight of male and female were observed relative to the dose of

Ag-NPs; however it has caused some alkaline and cholesterol changes which were assumed to be dose dependent [74]. Furthermore it was proposed that Ag-NPs do not cause any genetic changes in rats. Some scientists showed their concern while reporting the studies on toxicology of Ag-NPs to organism via skin [75]. Generally very limited research has been done on the issue highlighting the effect of Ag-NPs on the mankind and wild animals; further studies in this field are the need of the day; however Ahamed et al. have summarized the studies relating the issue in his review entitling the study of the impact of Ag-NPs on the environment [8] in which the authors have given detailed information relating various disorder caused by the Ag-NPs such as reproductive disorder and morphological deformation.

**5.3. Release of Ag-NPs from Functionalized Materials.** Consumer product comprised of any form of silver (Ag) is the main cause of silver release into the environment. Several articles have been published concerning the release of silver from wide range of materials that use silver nanoparticles (Ag-NPs) in their products such as functionalized fabrics paints [46, 76]. Usually inductively coupled plasma (ICP), energy dispersive spectroscopy (EDS), and transmission electron microscopy (TEM) are used for the detection of released silver from the materials to be investigated. In a report published by Lorenz et al., the authors hinted that with the growth of nanoindustry nearly all products contain nanoparticles especially sprays [76]. They used four different kinds of sprays for their experiments and confirmed the presence of nanoparticles. In one of the studies conducted by Benn and Westerhoff they reported that manufacturing techniques play a vital role in the release of silver (Ag) from the fabrics functionalized with Ag-NPs. They performed their experiment on socks that were made using different manufacturing techniques. The results obtained confirmed the presence of Ag-NPs in both the wash water and the socks. The presence of released silver (Ag) was confirmed in colloidal and ionic form and was considered to be an important factor in the release of Ag into the environment [46]. In a published article the authors conducted experiments for the assessment of release of Ag from the fabrics functionalized Ag-NPs. The authors revealed that the most important factor for Ag release from the Ag-NPs functionalized fabrics is the processes used for their incorporation into the fabrics during fabrication [50].

## 6. Mechanism of Action of Ag-NPs against Microbes

Silver (Ag) and especially silver nanoparticles (Ag-NPs) antimicrobial activity, irrespective of the strains of bacteria whether they are antibiotically resistant or not, is famous around the globe in scientific community [77]. The confirmed mechanism is yet to be discovered but can be related to the mechanism of silver (Ag) ions action on bacteria strains such as trypanosomes and yeasts, where accumulation of Ag-NPs occurs from the aqueous solution that ultimately causes saturation of enzymes and protein in the cell [77, 78]. In a published report the authors proposed the mechanism which states that the changes caused by the Ag-NPs in the cell wall

and nuclear along with DNA and RNA are the main cause of retarding the bacterial cell growth. Meanwhile Li et al. proposed three possible mechanisms that can cause bacterial cells death using Ag-NPs particle [48].

- (1) The first proposed mechanism states that the bacterial cell growth and proliferation are inhibited by the adhesion of Ag-NPs onto the cell wall (due to the fine particles size) of the bacteria thus causing changes in the cell wall in which intern is unable to protect the internal part of the cell [79].
- (2) In the second proposed mechanism the authors stated the penetration of the Ag-NPs into the bacterial cell causing changes in the DNA retarding its normal function thus ultimately causing its death. Silver nanoparticles penetrate through bacterial cell wall, resulting in DNA damage.
- (3) In the third proposed mechanism they stated that when Ag<sup>+</sup> ions interact with the proteins containing sulphur present in the cell wall of the bacteria this ultimately leads to the malfunctioning of the bacterial cell wall. This process is assumed to be the main mechanism in explaining the antibacterial activity [48, 79–82]. The various antimicrobial mechanisms have been depicted in Figure 4.

## 7. Ag-NPs Role in Medicine

**7.1. Antibacterial Properties.** The Ag-NPs are famous for its potent antibacterial activity against various strains of bacteria including highly pathogenic bacteria species (gram positive and gram negative bacteria) [83]. Sondi and Salopeck-Sondi investigated the antibacterial activities of Ag-NPs against *E. coli* on Luria-Bertani agar plates. The *E. coli* bacterial strains were used as representative species for gram negative bacteria. After the analysis of the obtained results the authors reported that the antibacterial activity of Ag-NPs against *E. coli* was dose dependent (concentration). At optimized experimental parameters, they found out that Ag-NPs were adhered to the cell wall of the gram negative bacteria (*E. coli*) that caused the destruction of the bacterial cell [84]. In another reported study scientists conducted experiments on the size related properties of the Ag-NPs on different species of gram negative bacterial strains [85]. The results obtained from their study suggested that size of Ag-NPs is an important factor in preventing the bacterial cells from their normal functions. Furthermore they also reported that smaller particle can easily adhere to the cell wall of the bacterial cells thus hampering their normal behavior such as permeability and respiration along with the release of the Ag ions from the Ag-NPs particle. Furthermore in another published study, researchers conducted experiments for elucidating the dose dependent properties of Ag-NPs on gram negative and gram positive bacteria; the authors reported that gram negative bacteria (*E. coli*) can be inhibited at relatively low concentration as compared to the gram positive bacteria (*S. aureus*) (see Figure 5). Shrivastava et al. revealed that the antibacterial activity of the Ag-NPs is both size and dose dependent; furthermore they also proposed

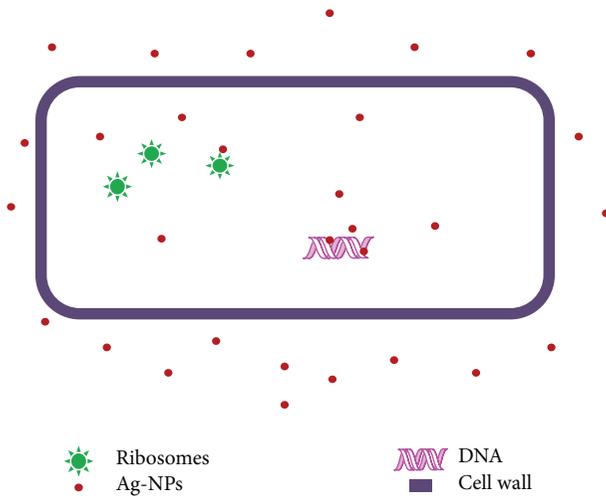


FIGURE 4: Schematic of antibacterial activity of Ag-NPs. Ag-NPs get adhered to the cell wall of bacterial cell through sulphur present in the protein comprising the bacterial cell wall, ultimately causing bacterial cell death. Ag-NPs also hamper protein synthesis along with directly causing damage to the interior of the bacterial cell by directly penetrating the cell wall and cell membrane.

the possible mechanism for the antibacterial activity of Ag-NPs, which states that the antibacterial activity of the Ag-NPs is governed by the adhesion and penetration pattern of the Ag-NPs into the cell wall of the bacterial cell, ultimately resulting in abnormal function [86]. In a study published by Pal et al., the authors revealed that the antibacterial activity of the Ag-NPs is structure (morphology) dependent [87].

**7.2. Antifungal.** Fungi are considered to play a vital role in causing fungal infections, especially in hospitals [88]. Along with the antibacterial activities of the Ag-NPs numerous studies had been reported on the antifungal activities of Ag-NPs, which reveals that Ag-NPs could be used as effective antifungal agent because Ag-NPs exhibit excellent antifungal properties against various species of fungi. In a report published by Kim et al. they tested the antifungal activities of the Ag-NPs against different fungal strains such as *Trichophyton mentagrophytes* (*T. mentagrophytes*) and *Candida albicans* (*C. albicans*) fungi and revealed that Ag-NPs exhibited good antifungal activity. Furthermore they also proposed the possible mechanism for the antifungal activity of Ag-NPs, which states that Ag-NPs cause abnormalities in the cell wall of the fungi which results in the abnormal functions (retarding the normal budding process) of the fungal cells (*C. albicans*) [10, 88, 89]. In another published paper researchers found out that catheters coated with Ag-NPs can result in complete inhibition of fungi (*C. albicans*). Recently, scientist reported the antifungal activities of Ag-NPs synthesized by tollens method. From the obtained results they revealed that the yeast cells proliferation can be inhibited with the help of Ag-NPs along without causing any harm to human fibroblastic cells. Another reported work states that Ag-NPs have exhibited antifungal activities against different strains of fungi such as *C. albicans* and *C. glabrata* *Trichophyton*

*rubrum* (*T. rubrum*) but the activity is dose dependent [90]. In short due to the reported literature available on the antifungal activity of Ag-NPs, it can be concluded that Ag-NPs can be used as antifungal agent against various strains (species) of fungi and can be helpful in overcoming various fungal infections caused by fungi.

**7.3. Antiviral Agent.** Recently, the increase in infectious diseases caused by virus such as SARS-Cov, influenza A/H5N1, influenza A/H1N1, Dengue virus, HIV, HBV, and new encephalitis viruses, is of prime concern. These infections can create havoc in no time because of the rapid proliferation (glimpses of destruction caused by these viral infections have been observed in some of the countries and the most dangerous of these viral infections were bird flu, swine flu, and dengue), ultimately resulting in causing severe damage to health and wealth of humans beings [91]. Ag-NPs are famous for their antimicrobial activities; therefore researchers have diverted their attention and started evaluating the importance of Ag-NPs in controlling infectious diseases caused by pathogens and viruses. However the number of reported works using Ag-NPs for controlling viral infections is very low but still it can pave the way for other researchers to show their interest in dealing against viral infections using nanoparticle specifically Ag-NPs. Elechiguerra et al. published their study conducted for assessing the effect of Ag-NPs on the HIV-1 virus. The authors concluded and revealed that the interaction between Ag-NPs and virus is size dependent (small sized nanoparticles are more effective against these viruses) [92]. They further enlightened the idea that Ag-NPs get adhered to the sulphur present in the gp120 glycoprotein knobs that results in hampering the normal activities of the virus therefore hindered the normal functions of the virus. This mechanism was second by another group of researchers when they published their report after assessing the role of Ag-NPs on HIV virus [92]. According to their published article they proposed that Ag-NPs were effective against HIV virus having the capability to bind to the sulphur present at gp120 glycoprotein knobs thus ultimately retarding their normal functions and binding to the hosts [10, 92].

Furthermore in a published article the authors reported that Ag-NPs play a vital role in inhibiting the synthesis of HBV RNA and extracellular virions *in vitro* (hepatitis B virus using HepAD38 cell line) [10, 93]. Sun et al. published their research work in which they conducted experiments on the PVP (polyvinylpyrrolidone) coated Ag-NPs in combination with protein for controlling the infection caused in HEp-2 cell by syncytial virus (RSV) [94]. They revealed that PVP coated Ag-NPs are effective in preventing the RSV virus infection. Furthermore they proposed the possible mechanism which stated that the PVP coated Ag-NPs bind to the G proteins present on the surface of viral cell thus suppressing the adhesion of the RSV virus cells to the host cells. Other researchers conducted experiment on Ag-NPs (having different size and geometry) and reported that Ag-NPs are very effective in inhibiting the normal functions of monkey virus [95]. De Gussemme et al. reported the usefulness of *bio*-Ag-NPs (biogenic Ag-NPs), and they concluded that both Ag-NPs and ionic Ag<sup>+</sup> play very important role in controlling

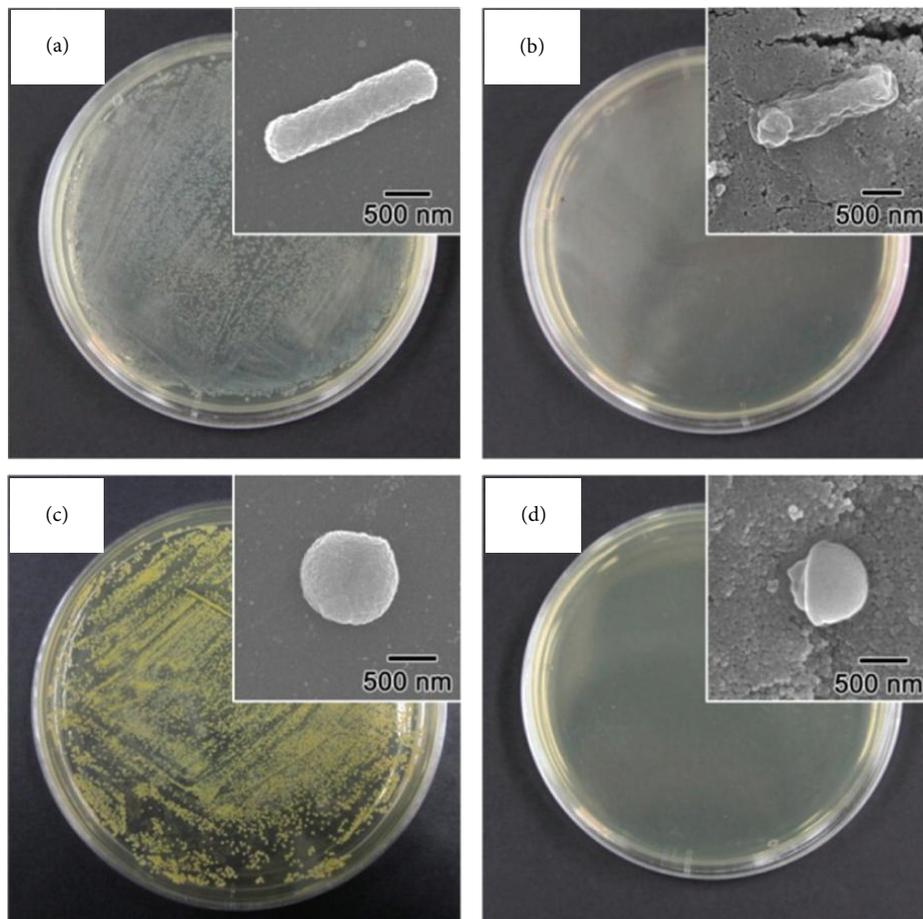


FIGURE 5: Photographs of colonies of (top) *E. coli* and (bottom) *S. aureus* (a, c) without nanoparticles treatment and (b, d) treated with  $\text{SiO}_2$ -Ag/PRh nanoparticles. Inset figures show the FE-SEM images of a single bacterial cell (left) in the absence of bactericidal agents and (right) treated with the as-prepared nanoparticles. Reproduce from Song et al. with permission from ACS publishers [19].

murine norovirus [96]. Xiang et al. conducted experiments for elucidating the inhibiting role of Ag-NPs against H1N1 influenza A virus. In the report the authors revealed that Ag-NPs are very effective in inhibiting the normal activity of H1N1 influenza A virus [97]. Furthermore they proposed that Ag-NPs can control the apoptosis of MDCK cells caused by H1N1 influenza A virus. In short almost all of the reported articles suggested the same mechanism which states that Ag-NPs get adhered to the outer proteins of the viral cells thus ultimately inhibiting the normal function of the viral cells. However authentic mechanism is yet to be established but Ag-NPs are considered to play a pivotal role in future for controlling infectious diseases caused by the viruses [98].

**7.4. Composite of Ag-NPs.** With the advancement in the field of nanotechnology in several engineered products such as clothing, respirators, water-filters, soaps, contraceptives, antibacterial sprays, and detergent and in numerous household products, silver (Ag-NPs) is used either in pristine form or in combination with other materials for various reasons especially in the biomedical field. With the number of products leveraging the benefits of silver, Ag-NPs have

various important applications in the area of medicine from wound dressing to control release of drug at targeted site. For example, Hameed et al. synthesized Ag-NPs silver and gold nanoparticles stabilized with cefuroxime (antibiotic). The composite materials showed various enzyme inhibition activities [99]. Furthermore pristine silver and composite of Ag-NPs have application in the area of electronics transparent conducting films, transparent electrodes for flexible devices, and flexible thin film tandem with solar cells. Several processing techniques have been reported by researchers for the preparation of pristine and composite of Ag-NPs for enhancing its application in various fields.

Usually composite of Ag-NPs is prepared by blending a wide range of natural and synthetic polymers or polymer coating over Ag-NPs which can play vital role in nourishing its various properties. Blended composite of Ag-NPs/polymer can be prepared by solvent casting method along with the use of more versatile technique such as electrospinning (used in the preparation of electrospun hybrid nanofiber scaffolds) [55, 83]. Li et al. reported that electrospun composite nanofibers comprised of PVA/chitosan/Ag-NPs can effectively be used as wound dressing agent due

to its proven effectiveness in inhibiting bacterial growth [100]. More recently Gurhan et al. proposed that by mimicking porous scaffold of collagen with electrospun composite nanofiber functionalized with Ag-NPs can efficiently inhibit bacterial growth. Moreover due to the structural similarities with the skin it can be used as potential scaffolds for skin tissue regeneration [101]. Furthermore electrospun composite nanofiber scaffolds were prepared by blending PEO-PCL/Ag-NPs. Stabilization of the Ag-NPs was achieved with the help of PEO [102]. Therefore electrospun composite nanofiber scaffolds functionalized with Ag-NPs are considered as an important asset in future medicine. Moreover Zeljka Jovanovic et al. prepared hydrogel containing PVP/Ag-NPs. They revealed that the nanocomposite hydrogel is capable of slow release of Ag, thus paving the way for its use as wound dressing [103]. In one of the published report, the authors unveiled a novel method for the synthesis of composite comprised of oleic-acid coated maghemite nanoparticles embedded in a 1,4-phenylenediamine/phosphotriazine matrix and silver nanoparticles covering the polymer surface. After the assessment of the magnetic properties of their composite, they concluded that the composite containing Ag-NPs has superparamagnetic properties, enabling its easy recovering with the help of external magnet field. The size of the silver nanoparticles (Ag-NPs) was less than 20 nm; finely dispersed Ag-NPs on the polymer surface added the antimicrobial properties to the composite material. Moreover Dallas et al. revealed that by modifying Ti/TiO<sub>2</sub> surfaces with citrate-coated Ag-NPs can efficiently exhibit antibacterial activity. Such kind of composite materials containing Ag-NPs with multifunctional properties has a high scope for use as antimicrobial agent. Furthermore they can also be used in targeted delivery of drug at specific site [104, 105].

**7.5. Scaffolds of Ag-NPs.** Nanoparticles specifically silver nanoparticles (Ag-NPs) are considered to be of prime importance due the emergence of antibiotic resistance bacteria [106]. These antibiotic resistant bacteria pose great threat to the human beings and their properties. Consumer products and devices containing silver are already in use in medicinal field such as prostheses, catheters, vascular grafts, and as wound dressings [107]. Silver (Ag-NPs) is very effective antimicrobial agents and plays a vital role in wound healing [108]. Silver nanoparticles (Ag-NPs) bind to the sulphur present in the protein comprising the cell wall of the microbes and phosphorous in the DNA, thus retarding their normal function. They also affect the bacterial respiratory system. Wide range of composite materials containing silver nanoparticles (Ag-NPs) has been prepared simply by blending them with various natural and synthetic polymers like cellulose, chitosan, polystyrene, acrylic acid [109], PCL, [110] gelatin, PVP [55], PEO [102, 111], and so forth. Furthermore other polymeric materials such as polyurethane, chitin, and its derivatives are widely used as wound dressings scaffolds in combination with Ag-NPs due to their famous antimicrobial and biocompatible properties [112, 113].

**7.6. Wound Dressing.** Wound dressings (bandages) functionalized with silver nanoparticles (Ag-NPs) are commercially

available and are frequently used for medicinal purpose for curing various infections such as burn wounds, toxic epidermal necrolysis, Steven-Johnson syndrome, chronic ulcers, and pemphigus [10]. In typical bandages (wound dressings) Ag-NPs are coated on the surface of polyethylene layer. Experiments have proven the superior wound healing properties of the Ag-NPs coated bandages (wound dressing) as compared to the wound dressing without Ag-NPs. Such wound dressing has the ability to prevent infections along with minimizing the healing time without any side effect [8]. In an article published on Ag-NPs, researchers concluded that bandages (wound dressing) containing Ag-NPs minimize the healing time of burns wounds. However no difference in healing time of deep burns wounds was found [8]. In a report published by research group, the authors proposed that dressing comprised of chitosan/silver nanoparticles (Cs/Ag-NPs) demonstrated superior healing rates contrary to the conventional wound dressing; furthermore they used chitosan as control in their experiments. Using Ag-NPs in wound dressing can be helpful in eradicating problems related to skin [20]. In one of the published report, researchers have incorporated Ag-NPs into chitin scaffolds which were later used as wound dressing agent. From their experiments they concluded that the wound healing and antimicrobial efficacy of chitin are further enhanced by the incorporation of Ag-NP [10, 112]. Furthermore in another published article researchers after their extensive study have revealed that hydrogel made of  $\beta$ -chitin and Ag-NPs can be effective in preventing various infection that can occur to wounds [114]. In another published article, researchers have reported that incorporating Ag-NPs (having size less than 20 nm) in guar gum, [110] a new cationic biopolymer has shown quicker healing and improved cosmetic appearance. Furthermore the biopolymer matrix functioned as hydrated surface that helped in cell proliferation. The antimicrobial activity of wound dressing functionalized with Ag-NPs is largely dependent on the concentration, release of silver along with the distribution and its degree of wettability [115]. The importance of Ag-NPs in bandages (wound dressing) has been acknowledged by the scientific community and can be helpful in paving the way for the fabrication of new materials containing Ag-NPs that can ultimately be used in future medicine [5].

**7.7. Implanted Materials.** Just before the beginning of the 21th century, artificial heart valve made of silicone (Silzone) coated with Ag-NPs was prepared for controlling endocarditis (an inflammation which occurs after surgery) [116]. The idea behind using Ag-NPs coating on the artificial silicone valve was to eliminate the dangers of microbial infections. Ag-NPs are considered to be an efficient coating agent on medicinal devices due to its proven antimicrobial activity. In one of the reported study researchers proposed that diamond-like carbon with Ag-NPs incorporated in the matrix can be useful if used as coating on the medicinal devices such as heart valves and stents [117]. Researchers also reported that the incorporation of Ag-NPs into poly(methyl methacrylate) ((PMMA) bone cement) can be helpful in hip and knee joint implantation; presence of Ag-NPs will give the added advantage of preventing microbial infections [118]. Ag-NPs are becoming

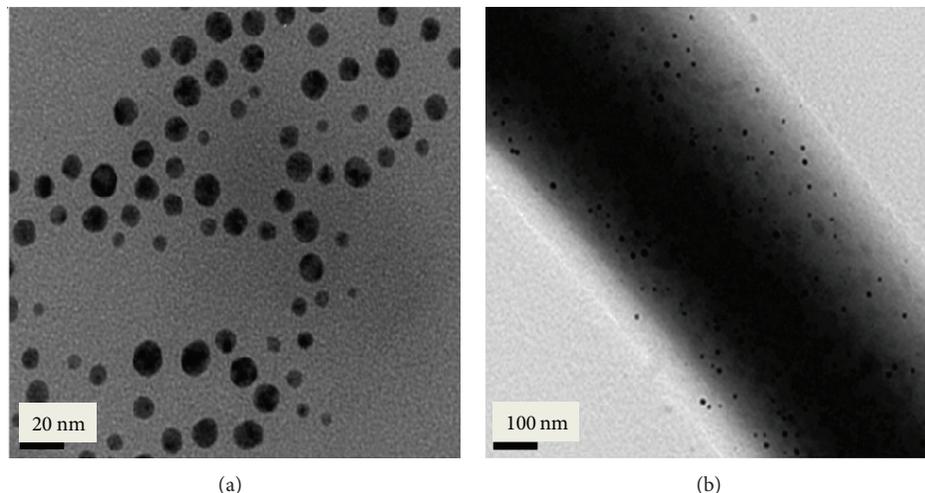


FIGURE 6: TEM images of (a) free silver nanoparticles and (b) silver nanoparticles distributed to the PHBV nanofibrous scaffolds (PHBV/Ag 1.0). Reproduce from Xing et al. with permission from ACS publishers [20].

important tool in surgery for preventing microbial infections; catheters impregnated with Ag-NPs are proving to be helpful in overcoming infections related catheters which are being used during surgery [119]. Xing et al. fabricated (poly-(3-hydroxybutyrate-co-3-hydroxyvalerate) PHBV nanofibrous scaffolds containing Ag-NPs. After extensive study on both osteoblast (bone cells) and fibroblast (skin cells) cultured on the PBHV/Ag-NPs hybrid nanofiber scaffolds they concluded that such scaffolds can be useful in bone and skin tissue regeneration (Figure 6) [20]. By fabricating the surface of structure of the bone implants devices and scaffolds with Ag-NPs it can overcome the risk associated with implantation surgery. Wu et al. concluded that the Ag-NP/nHA/TiO<sub>2</sub>/PA66 composite scaffold having porous structure with antimicrobial properties can be effective in control release of Ag at targeted bone implanted site. Apart from the antimicrobial activity the composite porous scaffolds with enhanced mechanical properties will provide favorable environment for adhesion and proliferation of the cultured cells [120]. Ye et al. conducted experiment for assessing the role of Ag-nHAnTiO<sub>2</sub>/PA66 membrane on MG63 osteoblast like cells *in vitro*. In their study they used nHA/PA66 and polytetrafluoroethylene (e-PTFE) membranes as control. From the obtained results the authors concluded that Ag-nHA-nTiO<sub>2</sub>/PA66 membrane had no adverse effect on the adhesion and proliferation of MG63 (osteoblast like) cells and the membranes simultaneously present tissue compatibility. Therefore Ag-nHA-nTiO<sub>2</sub>/PA66 membrane can be used for bone tissue regeneration [121]. Furthermore an extension of the work reported by Ye et al., Zhang et al. conducted experiments on rats and revealed that Ag-nHAnTiO<sub>2</sub>/PA66 membrane underwent osteogenesis process [122]. Saravanan et al. prepared CS/nHAp/Ag-NPs tricomposite scaffold; after conducting various experiments for the evaluation of its antimicrobial and biocompatible nature they revealed that the tricomposite CS/nHAp/Ag-NPs scaffold was effective in preventing bacterial infection. Furthermore the tricomposite (CS/nHAp/Ag-NPs) scaffolds

provided favorable environment for adhesion and proliferation of rat osteoprogenitor cells and human osteosarcoma cell lines [123]. Moreover Xie et al. published an article in which they proposed that Ti surface coated with HA/CS/Ag-NPs can be used as artificial bone implanting material due its multifunctional properties. Ag-NPs will act as antimicrobial agent. Chitosan will increase the biocompatibility and HA will help in providing native environment for the proliferation of the bone cells (osteoblastic cells; see Figure 7) [21, 123, 124].

## 8. Future Prospects

The importance of Ag-NPs in medicine is known to everyone. Ag-NPs have played pivotal role in inhibiting various infectious disease caused by microbes in combination with simultaneous helping in the burn wounds healing process, by preventing wound from infections caused by microbes. Furthermore Ag-NPs are also known for their anti-inflammatory and antiviral activity. Apart from its widely known antimicrobial activity it has remarkable application in biological field as well as in other research fields such as electrochemistry, biochemistry, nanoprism synthesis, garments, detergents and soap industry, involved in devising water purification system, and surgical instrument. Moreover Ag-NPs have a bright future in devices that can be used as artificial implants in future and minimizing the dependency on antibiotics. Recently researchers revealed novel biological application of Ag-NPs; the authors proposed that aqueous solution of Ag-NPs can be helpful in building new pharmaceutical and therapeutic agents. For example, in one of the published article the authors reported that aqueous solution of Ag-NPs can be used for curing the interstitial cystitis (inflammation of the bladder). They used mouse model for the assessment of the anti-inflammatory effect [119]. The full potential of this technology is yet to be investigated. A reliable mechanism responsible for the impressive biological activity of Ag-NPs is yet to be established and is considered to be a key factor

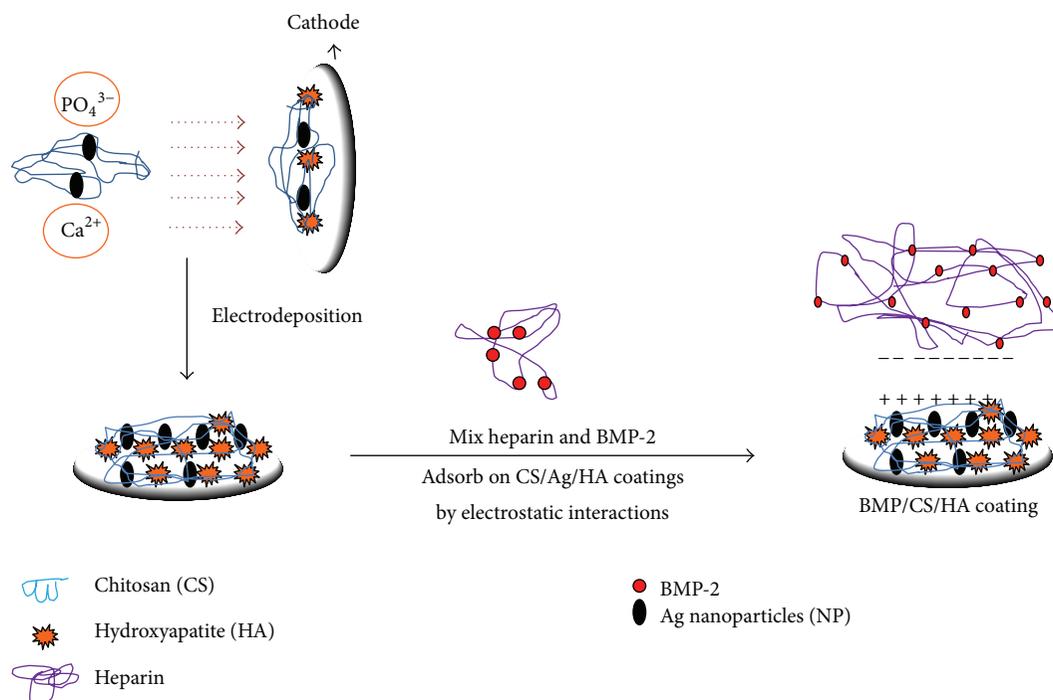


FIGURE 7: Titanium surface modified with growth factors, HA chitosan, and Ag-NPs. Reproduce from Xie et al. with permission from ACS publishers [21].

in future research. There is room for controlling the release of Ag and improving the stability of Ag-NPs used in various devices for various purposes especially in medicinal field. Finally, with the widespread use of Ag-NPs, addressing issue relating to the cytotoxicity is the cry of the day.

## 9. Conclusion

Ag-NPs are regarded as important addition in the area of nanomaterials because of the diversity it provides in terms of application in various walk of science. Due to their anti-inflammatory and antimicrobial activity Ag-NPs have diverted attraction of the masses towards themselves to be used as implanted material in artificial organs. Ag-NPs have proven it worth in inhibiting the microbial proliferation and microbial infection. Furthermore Ag-NPs have added a new dimension in the field of medicine concerning wound dressing and artificial implantation and in preventing post-surgical contamination caused by microbes. Apart from that Ag-NPs play a pivotal role and are considered as important ingredients in the preparation of commercially used products in industries.

## Conflict of Interests

It is hereby stated that the authors have no conflict of interests in publishing this paper.

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## Research Article

# Assessment of Size-Dependent Antimicrobial and Cytotoxic Properties of Silver Nanoparticles

Yoon Jeong,<sup>1,2</sup> Dong Woo Lim,<sup>1,2</sup> and Jonghoon Choi<sup>1,2</sup>

<sup>1</sup> Department of Bionano Technology, Hanyang University, Seoul 133-791, Republic of Korea

<sup>2</sup> Department of Bionano Engineering, Hanyang University ERICA, Ansan 426-791, Republic of Korea

Correspondence should be addressed to Jonghoon Choi; [jonghchoi@hanyang.ac.kr](mailto:jonghchoi@hanyang.ac.kr)

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Nanoscale silver has been increasingly applied to commercial products for their antimicrobial function as antibiotics and disinfectants. In this work, the different sizes of silver nanoparticles (AgNPs) were studied not only in *Methylobacterium spp.* for their antimicrobial potential but also in human peripheral blood mononuclear cells (PBMCs) for their cytotoxicity in order to determine responses dependent on their particle size. Size controlled silver particles were prepared by chemical reduction of silver cations ( $\text{Ag}^+$ ) and then dispersed in water for their physicochemical characterization using transmission electron microscopy (TEM), dynamic light scattering (DLS), and zeta potential measurements. To ascertain antimicrobial response, water-soluble silver nanoparticles were mixed into *Methylobacterium spp.* cultured for two days and the sample from the broth was spread on the agar plate for colony counting. 10 nm nanoparticles showed more antimicrobial activity than 100 nm particles at which concentrations were equivalently controlled. Increased cytotoxic effect of smaller silver nanoparticles was also observed in PBMCs cocultured with particles. Silver ions released from 10 nm particles might be correlated with upregulated antimicrobial and cytotoxic properties of AgNPs.

## 1. Introduction

Lethal thermal burning and critical infections were a major concern of death in the medieval ages. Since then, silver has been introduced and obtained attention for centuries in health care because of their growth inhibitory capacity against microorganisms. In 1960s, silver sulfadiazine was first used, as an antimicrobial compound, to cure burn patients [1–3] and has also been used for a long time to deal with various diseases like cauterization, pleurodesis, and skin wounds [1, 4, 5]. The silver nitrate solution often called as “Credé’s prophylaxis” that is preventive treatment using the instillation of silver nitrate solutions into the conjunctiva of newborns is now regarded as a standard practice in obstetric studies, wound healing applications [6, 7], and biomaterial developments [8, 9]. Because of its fluent antimicrobial action, the number of reported silver usages in the environment as well as in the body has been increasing. Their effect of boosting the number of white blood cells, for example, was demonstrated already [10].

Nanotechnology has opened new and improved modalities of AgNPs in biomedical applications. Compared with macro- or microscale silver materials, nanoscale silver is able to readily release  $\text{Ag}^+$  ions. Before the nanostructured silver is introduced, the applications of silver products have been considerably limited due to their potential side effects which resulted from material defects, impurities, and so forth. Nanotechnology currently is employed as a tool for producing highly purified and well-defined AgNPs, and it has greatly helped to minimize the possible side effects. Enhanced antimicrobial function of AgNPs has promoted active application and commercialization of them in a wide variety of fields and products: catheters, bandages, water filtering, socks, toothpaste, and so forth ([11], see [12] for a database).

Toxicological studies of silver products have been reported actively and scrutinized their safe usages [13–17]. Literatures have been discussing the biological response of AgNPs since year 2000 [13, 14]. The essence of those studies is that the size of AgNPs could be an important factor in

that smaller particles would have larger surface area where it can potentially release more silver ions as a key player for observed toxicity. Also, there is a necessity to demonstrate whether smaller particles have the easier route of endocytosis and exocytosis into cellular membranes and in or out of tissue, blood vessels, and so forth [18–20]. Since the possible mechanisms of the antibacterial property of nanoscale silver has not been clearly shown, however, there is an unmet need to address questions; what the safe range of concentration would be applied to animal/human model, and what the key factors would be to kill bacteria efficiently while maintaining their biocompatibility.

Since blood compatibility might be the first requirement for any biomedical device and for commercial products including AgNPs, here, we report assessment of cytotoxicity of AgNPs which interacted with human PBMCs following physicochemical characterization of prepared AgNPs. We cocultured AgNPs with human PBMCs and continuously observed the number of dead cells which resulted from the constant interaction. Since a particle size might be one of the most significant factors affecting particle interactions with cells, we prepared two different sizes of AgNPs (10 and 100 nm in average diameters) with identical citric acid-terminated surface. Different concentrations of those nanoparticles were evaluated to the PBMCs respectively, and then, the maximum concentration showing biocompatibility to the PBMCs was obtained, empirically. In order to probe antimicrobial function of the AgNPs that have been previously demonstrated for their safety to human blood cells, we introduced particles to the *Methylobacterium spp.* and assayed the inhibition responses of AgNPs dependent on different sizes and concentration.

## 2. Experimental Methods

**2.1. Silver Particles Preparation.** Sodium citrate and silver nitrate were obtained from Sigma-Aldrich. AgNPs were prepared by the well-known Turkevich method [21, 22]. A solution of silver nitrate ( $1.0 \times 10^{-4}$  M) was heated and the droplets of sodium citrate were added to the solution. The reduction of silver ions occurred as monitored by the solution color change to yellow. Less amount of sodium citrate was added (0.05%) to the silver nitrate solution to obtain larger particles. The citrate ions act as both a reducing agent and a capping agent resulting in the citrate terminated AgNPs.

**2.2. Characterization.** The AgNPs were characterized by TEM and DLS measurement. TEM and high-resolution TEM (HRTEM) images were obtained using a JEOL JEM-2100F microscope. The TEM samples were prepared by drying a droplet of the AgNPs suspension on 300 mesh-size carbon film-coated copper grid. The size and zeta potentials of nanoparticle suspension were measured using DLS (Zetasizer Nano, Malvern, Westborough, MA).

**2.3. Cytotoxicity Test.** The effects of AgNPs on the viability of PBMCs (ATCC co.) were evaluated using a LIVE/DEAD viability/cytotoxicity kit. Human PBMCs were plated in 6 well

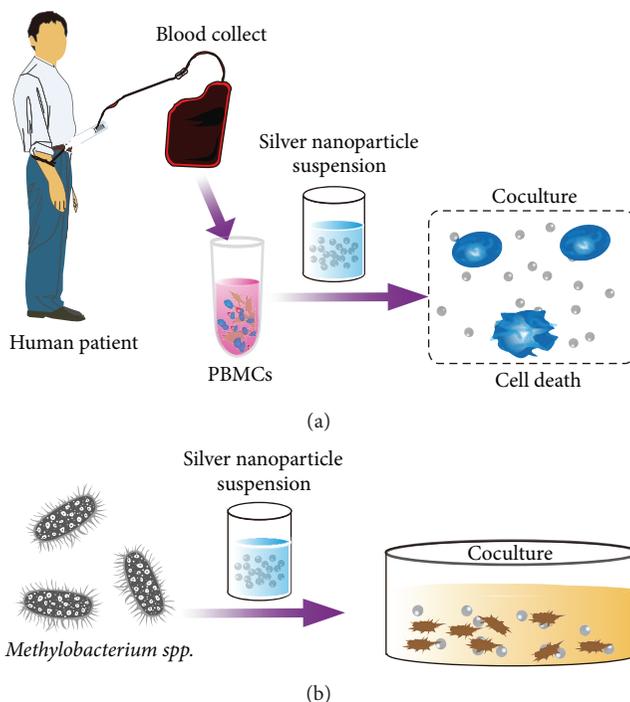


FIGURE 1: (a) Schematic representation of *in vitro* cytotoxicity test and (b) antibacterial property assay of AgNPs.

plates ( $1 \times 10^5$  cells per well) in RPMI supplemented with 10% (v/v) fetal bovine serum and 1% sterile antibiotics. The cells were cultured at  $37^\circ\text{C}$  in a humidified atmosphere of 5%  $\text{CO}_2$ /95% air for 8 h–12 h. Subsequently, untreated control, DMSO, and 0.1 to 1.0 mg/mL AgNPs were added to the cells in serum free medium and cultured for 8 hours. Results are quantified as relative values to those of the negative control.

**2.4. Antibacterial Tests.** The antibacterial activity of the AgNPs was evaluated against *Methylobacterium spp.* (KCTC 12618, South Korea) bacteria. A  $100 \mu\text{L}$  volume of each cultured bacteria was inoculated in R2A broth (MBcell, U.S.A.) and subsequently incubated with untreated control, methanol, and 0.1 to 1.0 mg/mL AgNPs. After incubation under shaking conditions (180 r.p.m.) at  $30^\circ\text{C}$  for 24 hours,  $100 \mu\text{L}$  triplicate samples of decimal dilutions were spread on R2A agar (MBcell, U.S.A.) plates. After incubation at  $30^\circ\text{C}$  for 48 h, the colonies on the plates were counted.

**Statistical Analysis.** One-tailed Mann-Whitney  $U$  test was performed using GraphPad Prism (version 5 for Mac OS X, GraphPad Software, San Diego, CA, USA, <http://www.graphpad.com/>).

## 3. Results and Discussion

In Figure 1, we showed a schematic drawing of assay procedures for *in vitro* cytotoxicity and antimicrobial properties of AgNPs. The possible mechanism for cytotoxicity of AgNPs would be that the intact of particles on the cell

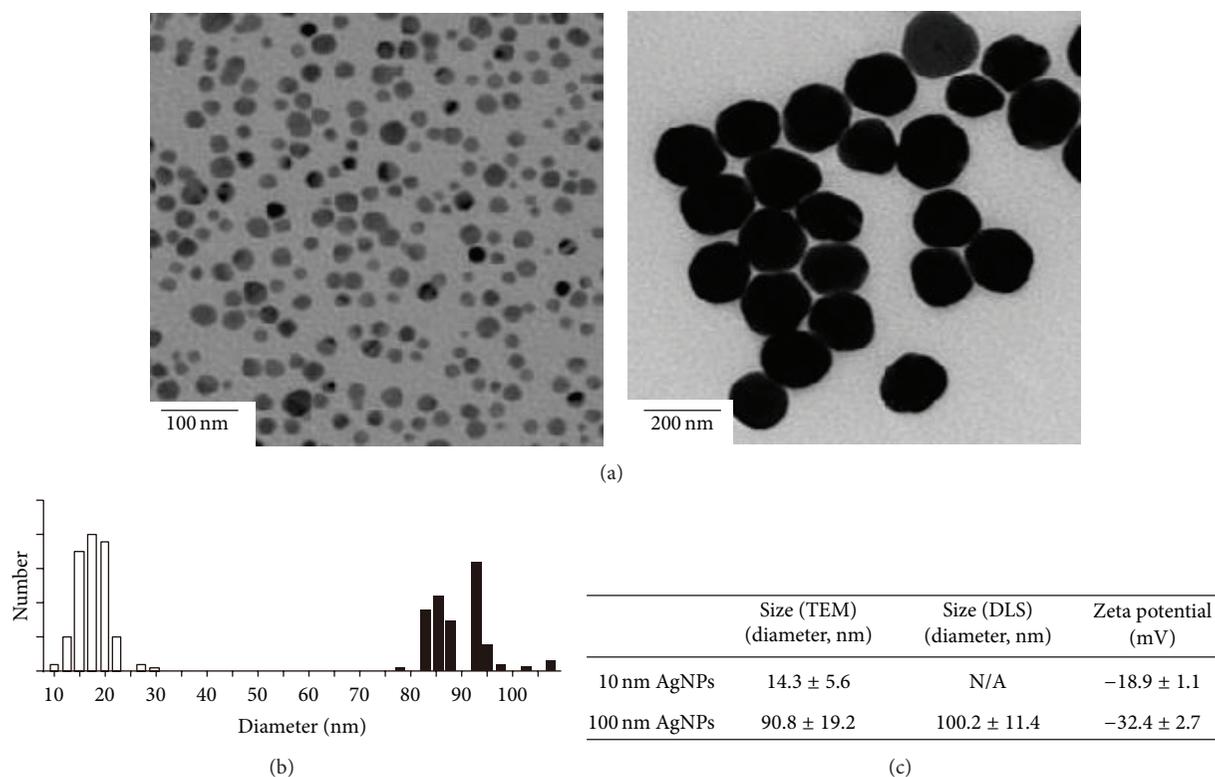


FIGURE 2: Size and shape of AgNPs measured by TEM and DLS (a–c). Comparison of nanoparticle sizes obtained by two different measurement techniques, image analysis of the particles' TEM results and DLS of particle suspension. Smaller nanoparticles (< 10 nm) can be observed in TEM image analysis results ((a), left) as well as in DLS result ((b), white bars). Larger particles are also shown in a TEM result ((a), right) and in DLS spectra ((b), black bars). (c) Summaries of the physicochemical characteristics of AgNPs tested in the study. Polydispersity indexes of two different size particles were similar at the values of 0.21 for 10 nm and 0.32 for 100 nm AgNPs. Size of particles varies depending on the measurement technique applied. Strong negativity of zeta potentials for AgNPs solution is because of the citrates on the surface of them.

wall subsequently ruptured the cell membrane or that silver ions blocked the ion channels of cells. Also, it would be possible that AgNPs and their released silver ions promote denaturation, which cleaves disulfide bonds in cell surfaces, or oxidation of cells. These processes may be responsible for the causes that the cell (or bacteria) loses its control on exposure to the oxidation reactions. Therefore, AgNPs generate reactive oxygen species in water that would degrade the integrity of cell walls.

We prepared different sizes of AgNPs for their effects and each particle was characterized first to compare their differences in physicochemical properties. The size and shape of AgNPs are studied by the TEM. Representative images taken from TEM are shown in Figure 2(a) (Left: 10 nm AgNPs, Right: 100 nm AgNPs). Image analysis of TEM taken for nanoparticles reveals the average diameters of two preparations of AgNPs (Figure 2(a)). To confirm the size distribution and mean values observed from TEM, the measurement of particle sizes was also performed in water by using a DLS (Figure 2(b)). Figure 2(c) describes a comparison of nanoparticle sizes between two preparations of AgNPs. Analogous point between two measurement techniques is that they have similar results in particles' mean diameter. However, there are also differences between two methods.

First, smaller particles in nanoparticle suspensions (<10 nm) may only be seen in TEM. In TEM image analysis, each particle recognized by software can be recorded for their size and registered for their presence. However, in DLS, particles should be able to scatter the light strongly enough to be read by a detector. If the sample is polydisperse, weak light scattering particles (smaller particles) would not be seen by DLS because strong light scattering particles (larger particles) will mask any weak scatterings. Nevertheless, DLS is useful to quickly measure mean size of particles since TEM image analysis usually requires a massive work of image processing. Furthermore, one cannot ignore that the particles in TEM image may not represent the entire samples (biased in a small region). Since each method has its own merits and demerits, one should not rely on the single method of characterization to confirm the mean size of nanoparticles.

In order to study the effects of citrate-covered surface of AgNPs, zeta potentials of each particle were measured in water. The citrated terminated AgNPs would be deprotonated in neutral water carrying enough surface charges to disperse each other. Citrate is a conjugate base of citric acid (2-hydroxypropane-1,2,3-tricarboxylic acid) which is a common surfactant for nanoparticles to prevent them from aggregation by providing electrostatic force. It also helps particles to

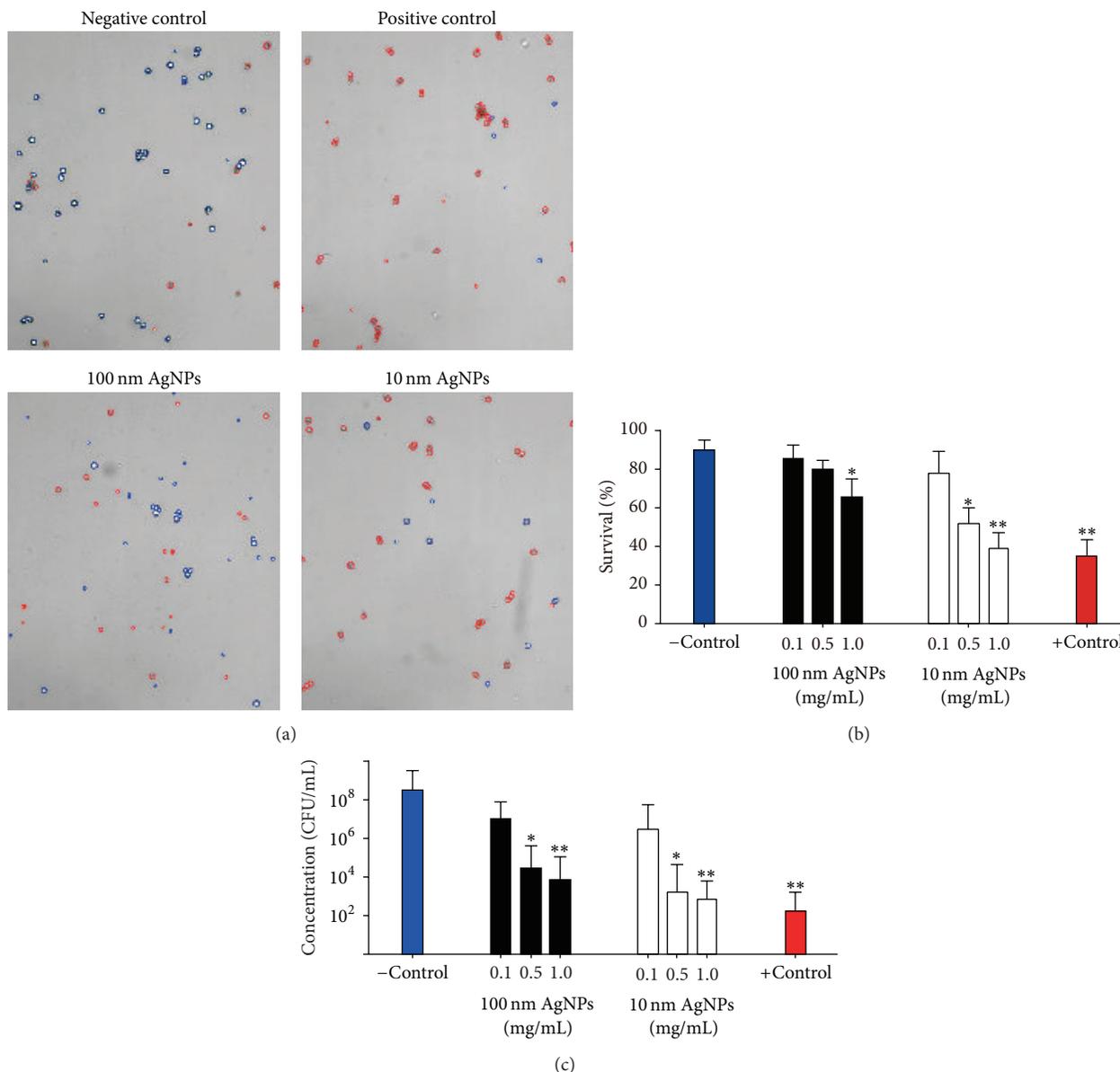


FIGURE 3: Cytotoxicity test of AgNPs to PBMCs and antimicrobial effect to *Methylobacterium spp.* (a) The numbers of live (blue) and dead (red) cells were counted for each sample. (b) 10 nm AgNPs displayed increased cytotoxicity in 0.5 mg/mL concentration between two different size particles. 1.0 mg/mL of 10 nm AgNPs showed the highest cytotoxicity to PBMCs comparable to the one from the positive control (DMSO). (c) Antimicrobial test of AgNPs against *Methylobacterium spp.* Both 100 and 10 nm AgNPs displayed strong antimicrobial effects in 0.5 mg/mL or higher concentration coincubated with the bacteria. 1.0 mg/mL of 10 nm AgNPs showed the equal killing efficiency as to the positive control (methanol). \*  $P < 0.05$ , \*\*  $P < 0.01$ , one-tailed Mann-Whitney  $U$  test.

be dispersed better in water and to protect the core material from degradation or decomposition.

Figure 2(c) summarizes the size and zeta potential values of each sample. The highly negative zeta potential ( $-18.9 \sim -32.4$  mV) for both preparations of AgNPs in water is due to citrates on the particle surface deprotonated in neutral water.

Figure 3 summarizes the cytotoxicity and antimicrobial activity caused by AgNPs to human PBMCs and *Methylobacterium spp.* These results demonstrated that the cytotoxicity

was clearly depending on the concentration of nanoparticles added in the cell culture (Figures 3(a) and 3(b)). Antibacterial tests were performed against *Methylobacterium spp.* with different concentrations of AgNPs (from 0.1 to 1 mg/mL) (Figure 3(c)). In the negative control, *Methylobacterium spp.* have average number of  $1 \times 10^8$  colonies, while the positive control only contains  $4 \times 10^2$  colonies. A similar number of colonies as that of the positive control are registered in groups with 1.0 mg/mL of both 10 and 100 nm AgNPs.

Although slightly higher antimicrobial properties of AgNPs are observed in 10 nm particles, 1.0 mg/mL AgNPs for both particles is selected as an optimal concentration that can statistically hinder the growth of *Methylobacterium spp.* as good as the positive control does.

Between two different size preparations of AgNPs, smaller particles (10 nm average in diameter) showed a higher cytotoxicity when two particles were compared in the same concentration. Smaller particles have a larger surface area when the concentrations of two groups are equally maintained. Large surface area could promote more interactions between particles and cells as well as faster release of silver ions. Citrates on the surface of AgNPs may prevent particles from direct, physical contact with cells in smaller surface area ( $<10\text{ cm}^2$ ) but it may not keep up with the increasing silver ion release in larger surface areas ( $>10\text{ cm}^2$ ) resulting in that cytotoxicity of 1.0 mg/mL of 10 nm AgNPs becomes the highest. Larger surface area also provides higher reactivity of particles and better chances of interacting with solvent ions that will eventually draw more ions from particles.

It has been reported that the silver ions are responsible for AgNPs' antibacterial properties [23–25]. It is an open question whether cytotoxicity happened in an analogous way by the silver ions. Silver ions are able to bind to any sulfhydryl group of the cell wall. Sulfhydryl groups especially in the enzyme systems can be degraded by these silver ion conjugations. Conjugated silver ions can prevent cells from a normal energy and electron transport [23–25]. It is also reported that silver ions can damage bacteria's respiratory chain by conjugating with proteins responsible for it. More studies are required to fully understand whether PBMCs can be lysed by the similar mechanisms that silver ions do for microorganisms.

Further questions regarding the cytotoxicity and antimicrobial property caused by AgNPs are first clarifying which proteins of cells' enzyme system are responsible for conjugation with silver ions. Anion exchanger 1 (AE1) which is transporter of chloride and bicarbonate across plasma membrane and glycophorin C (GYPC) which is the membrane protein of erythrocytes are examples of protein responsible for binding with silver ions released from AgNPs. Another question will be whether other forms of silver (e.g., silver plates) could also cause the similar effects as observed from AgNPs. In order to probe that, systematic study of silver ion release from various morphologies of silver materials should be preceded. In many cases, the metallic silver materials have a poor solubility so that the silver ions released from them could be scarce. However, any new morphology of silver materials introduced by nanotechnology should also be tested for their silver ion release.

In this study, we focused on the role of  $\text{Ag}^+$  ions in antimicrobial and cytotoxic activities but we may further investigate whether the particles by themselves are responsible for a death of PBMCs. Localization of AgNPs inside the cells may answer this issue since PBMCs are notorious for their insufficient cellular systems prepared for an endocytosis. Internalized particles by a membrane rupture would be

strong evidence that the PBMCs are lysed by Ag particles. In the same sense, lactate dehydrogenase (LDH) assay would be additional tool for probing cytotoxicity by marking the presence of LDH enzyme diffused out of membrane-degraded PBMCs in the solution with AgNPs. Finally, the observed cytotoxic properties of AgNPs should be carefully analyzed in a scale of either human or small animals' body weights to correctly predict their health risks. Recent studies about AgNPs coated catheters did find a significant release of silver ions in the course of time but the total amount calculated in a body weight was small enough to be negligible for causing a health risk [25].

#### 4. Conclusion

Two different size preparations of AgNPs were prepared and assayed for their antimicrobial and cytotoxic properties. 10 nm AgNPs displayed more cytotoxic and antimicrobial effect compared to 100 nm sized particles with similar surface chemical composition and the same concentration. A concentration-dependent effect on both cytotoxic and antimicrobial function was observed with substantive killing efficiency occurring at the highest nanoparticle concentrations ( $>0.5\text{ mg/mL}$ ). These data suggest that the average size of AgNPs is a key factor and blood-contact medical devices and commercial products that contain AgNPs should be carefully monitored for the size and amount applied and their correlating potential safety concerns.

#### Conflict of Interests

The authors declare that there is no conflict of interests regarding the publication of this paper.

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## Research Article

# A Study of Antibioactivity of Nanosilver Colloid and Silver Ion Solution

**Kuo-Hsiung Tseng, Heng-Lin Lee, Der-Chi Tien, Ya-Lan Tang, and Yi-Syuan Kao**

*Department of Electrical Engineering, National Taipei University of Technology, Taipei 10608, Taiwan*

Correspondence should be addressed to Kuo-Hsiung Tseng; [khtseng@ee.ntut.edu.tw](mailto:khtseng@ee.ntut.edu.tw)

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The colloidal silver solution was successfully prepared in dielectric fluid by using electrical spark discharge (ESD) without any surfactants. It does not require the toxic chemical agents in the process, which may affect the effectiveness of nanosilver colloid as an antibacterial agent. Nanocolloidal silver produced by ESD is characterized as low cost, zero environmental pollution, continuous, and rapid mass production process. In order to test the effect of antibioactivity, nanosilver dough was tested; the silver nanofluid was prepared by ESD machine, made into dough at different concentrations, and fermented for three hours in order to observe changes in the diameter of the dough. The results showed that the effect of effectiveness of nanosilver at the concentration of 100 ppm was weak, whereas the effect of 60 ppm silver ion (100 ppm  $\text{AgNO}_3$ ) was significant, as the dissociation rate of silver ion concentration correlates to the antibioactivity.

## 1. Introduction

Using ESD to prepare nanoproductions is a feasible method, which can process super hard materials (electrodischarge machining equipment) in the earliest stages. Traditional electric discharge machining (EDM) places the tool electrode and work piece in an insulating working fluid and applies DC voltage to the two poles. In recent years, nanotechnology has developed rapidly, and the research of ESD has focused on the characteristics and applications of nanoparticles or fluid in dielectric fluid [1]. For ESD processing, a set of continuous preparation methods has been developed [2–4], which can effectively be applied to mass production and further developed toward nanotechnology applications. The heat generated by ESD melts the surface of the electrode into metal particles or charged ions and then sputters and dissolves them in the dielectric fluid [5].

As a result of the abuse of antibiotics, bacterial resistance has continued to increase, thereby decreasing the antibiotic potency. Hence, efforts have been made in seeking alternative traditional antibacterial materials. Among the new antibacterial materials, silver nanoparticles and silver ion are the rising stars for resisting bacteria in the postantibiotic era, especially

silver ion, which dominates sterilization. Recent studies have found that some metal ions have oxidative antibiosis, and the descending order of oxidizing power is  $\text{Ag} > \text{Cu} > \text{Cr} > \text{Ni} > \text{Zn} > \text{Fe}$ . As positively charged silver ion has the best reducing force, it is oxidized by oxygen (O) to generate oxygen free radicals with a bactericidal mechanism. Silver is an efficient antibacterial material that has long been studied [6–8], as it can inhibit fungi, viruses, and bacteria [9–15]. Literature [16] found that 20 ppm silver ion is required to fight *Staphylococcus aureus*. In biomedical materials, silver nanoparticles can dissociate silver ions [17–21] and enter cell membranes at low concentrations, thus reducing the enzyme activity in bacteria and obstructing biochemical synthesis to result in antibacterial action.

## 2. Research Method and Process

*2.1. Preparation of Nanosilver by Electrical Spark.* The metal nanoparticles were chemically prepared previously, with an added suspending agent to control nanoparticle size and concentration. This study used ESD to split silver material into nanosized particles by arc discharge. The process was

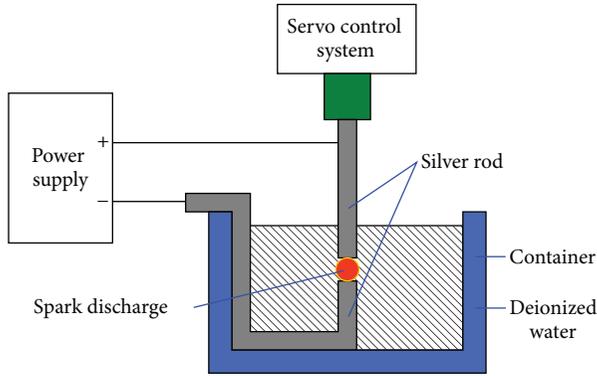


FIGURE 1: ESD nanosilver preparation system framework.

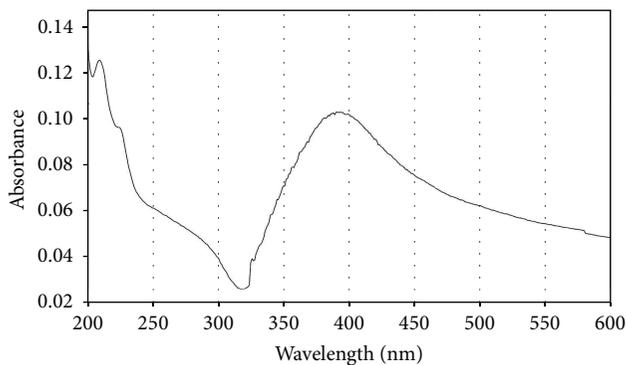


FIGURE 2: UV absorption spectrogram of water phase nanosilver.

free from chemical agents. Only the Pure solutions were prepared such as deionized water or alcohol as the medium. The silver nanoparticles suspending in the dielectric fluid were prepared by setting voltage and current parameters and selecting  $T_{ON}$ - $T_{OFF}$ . Easy and mass production is available without pollution, which contributes greatly to the preparation of nanomaterials. However, the characteristics of nanocolloid are closely related to the electrical discharge parameters. During discharge the voltage, current, dielectric fluid, and ambient temperature influenced the concentration of nanosilver colloid and particles size. And silver rod with purity of 99.9% was selected as the target. The system framework is as shown in Figure 1.

## 2.2. Silver Nanofluid Product Analysis

**2.2.1. UV-Vis.** UV absorption spectrogram analysis shows the light, among all wavebands from UV to infrared, has resonant effect with water phase nanosilver particles. As different materials have different resonance spectra, the resonance spectrum can be effectively used to prove and analyze for silver material. As shown in Figure 2, the resonance point of water phase nanosilver is at 393 nm.

**2.2.2. Nanosizer.** The particle distribution of nanofluid was measured by ZS-90 equipment by the Malvern Company. Using this equipment the particle distribution of nanofluid

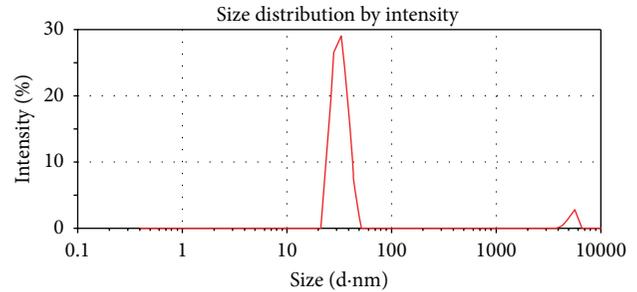


FIGURE 3: Particle size distribution of water phase nanosilver.

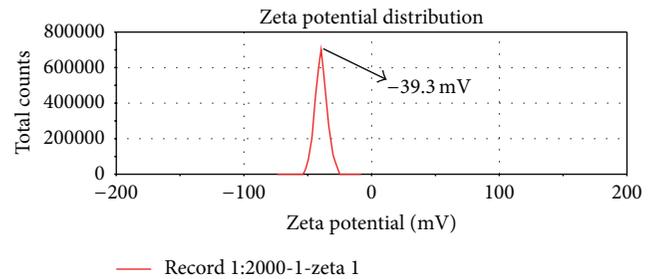


FIGURE 4: Surface potential of water phase nanosilver.

can be known within a few minutes. Figure 3 shows the analysis result of water phase nanosilver prepared by ESD. This equipment is useful in this research due to rapid analysis and low cost.

**2.2.3. Surface Potential Analysis.** According to the surface potential in Figure 4, the silver nanoparticle surface had  $-39.3$  mV voltage, and this voltage determined the dispersibility and suspending power of the silver nanofluid. The produced nanoparticles were nanoscale particles; however, under interparticle Van der Waals force and universal gravitation, any particles approaching each other would gather into large particles, which sink when attracted by universal gravitation.

When the surface potential of nanoparticle was greater than 30 mV. Exhibit good dispersibility and suspending power. Therefore, silver nanofluid produced by the electric spark method had  $-39.3$  mV voltage, with excellent suspending and dispersion power, and can be preserved for the long term at normal temperature and pressure.

**2.2.4. SEM and EDX.** The SEM photo shows the silver nanoparticles in pure water with particle size of about 20 nm, as shown in Figure 5. Then in the EDX component analysis, as shown in Figure 6, the particles were composed of nanosilver.

## 3. Experimental Results and Discussion

Both the nanosilver colloid by the ESD and  $AgNO_3$  solution is prepared for making the comparison of antibioactivity potency test. Two tests were conducted: (1) experiment on

TABLE 1: Experimental result of antibioactive effect of nanosilver.

Dough diameter	Dough composition				
	Water (5 g) + yeast (4 wt%) + Nanosilver (0 ppm)	Water (5 g) + yeast (4 wt%) + Nanosilver (20 ppm)	Water (5 g) + yeast (4 wt%) + Nanosilver (40 ppm)	Water (5 g) + yeast (4 wt%) + Nanosilver (80 ppm)	Water (5 g) + yeast (4 wt%) + Nanosilver (100 ppm)
Before fermentation (cm)	3.2	3.3	3.4	3.1	3.2
After fermentation (cm)	4.5	4	4	4.5	4.5

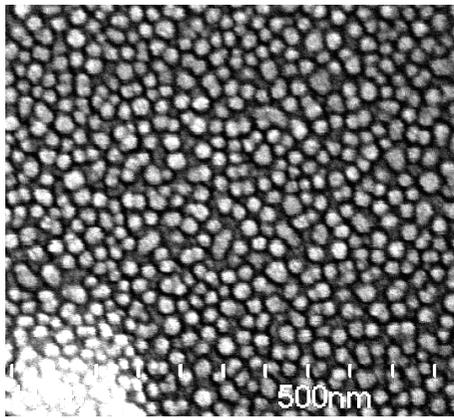


FIGURE 5: SEM photo of nanosilver prepared using pure water.

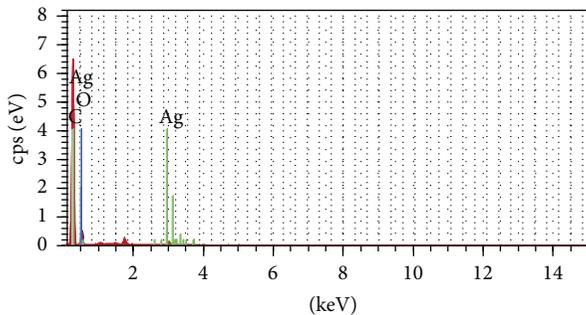


FIGURE 6: EDX component analysis of nanosilver prepared using pure water.

antibioactivity of nanosilver for yeast and (2) experiment on antibioactivity of  $\text{AgNO}_3$  for yeast.

**3.1. Experiment on Antibioactivity of Nanosilver for Yeast.** As moisture evaporates during fermentation, a petri dish with a cap was placed on the top of the dough to guarantee weight accuracy. Nanosilver at different concentrations was added in the dough and compared with dough without nanosilver. The result is as shown in Figure 7: (a) before fermentation of dough with only water and yeast, (b) after 3-hour fermentation of dough with only water and yeast, (c) before fermentation of dough with 20 ppm nanosilver, (d) after 3-hour fermentation of dough with 20 ppm nanosilver, (e) before fermentation of dough with 40 ppm nanosilver,

TABLE 2: Experimental result of antibioactivity effect of  $\text{Ag}^+$ .

Dough diameter	Dough composition		
	Water (5 g) + yeast (4 wt%) + $\text{Ag}^+$ (0 ppm)	Water (5 g) + yeast (4 wt%) + $\text{Ag}^+$ (60 ppm)	Water (5 g) + yeast (4 wt%) + $\text{Ag}^+$ (120 ppm)
Before fermentation (cm)	3.3	3.2	3.1
After fermentation (cm)	4.8	3.4	3

(f) after 3-hour fermentation of dough with 40 ppm nanosilver, (g) before fermentation of dough with 80 ppm nanosilver, (h) after 3-hour fermentation of dough with 80 ppm nanosilver, (i) before fermentation of dough with 100 ppm nanosilver, and (j) after 3-hour fermentation of dough with 100 ppm nanosilver.

It is found that the four groups of dough had similar degrees of fermentation, with the comparison as shown in Figure 8 and Table 1. Moreover, the antibioactive potency was poor, which may be due to insufficient dissociation rate of silver atom released from silver nanoparticle.

**3.2. Experiment on Antibioactivity of  $\text{AgNO}_3$  for Yeast Fermentation.**  $\text{AgNO}_3$  exhibit high ionic mobility in water solution due to high soluble property. The experiment on the antibioactivity of dough with  $\text{AgNO}_3$  for the yeast is as shown in Figure 9, (a) dough without  $\text{AgNO}_3$  and with yeast, (b) after 3-hour fermentation of dough without  $\text{AgNO}_3$  and with yeast, (c) dough with 100 ppm  $\text{AgNO}_3$  and yeast, (d) after 3-hour fermentation of dough with 100 ppm  $\text{AgNO}_3$  and yeast, (e) dough with 200 ppm  $\text{AgNO}_3$  and yeast, and (f) after 3-hour fermentation of dough with 200 ppm  $\text{AgNO}_3$  and yeast. The results showed that while dough with 100 ppm  $\text{AgNO}_3$  was still fermented, its diameter increased by only 0.2 cm, suggesting that the antibioactivity of  $\text{Ag}^+$  (60 ppm) was significant. Dough with 200 ppm  $\text{AgNO}_3$  was not fermented; the reason is that the 120 ppm  $\text{Ag}^+$  had killed the yeast completely. Figure 9 and Table 2 show the comparison, suggesting that the silver ion has excellent antibioactivity effect.

The experimental results show that, in the antibioactivity of nanosilver colloid prepared by ESD, the  $\text{Ag}^+$  was more

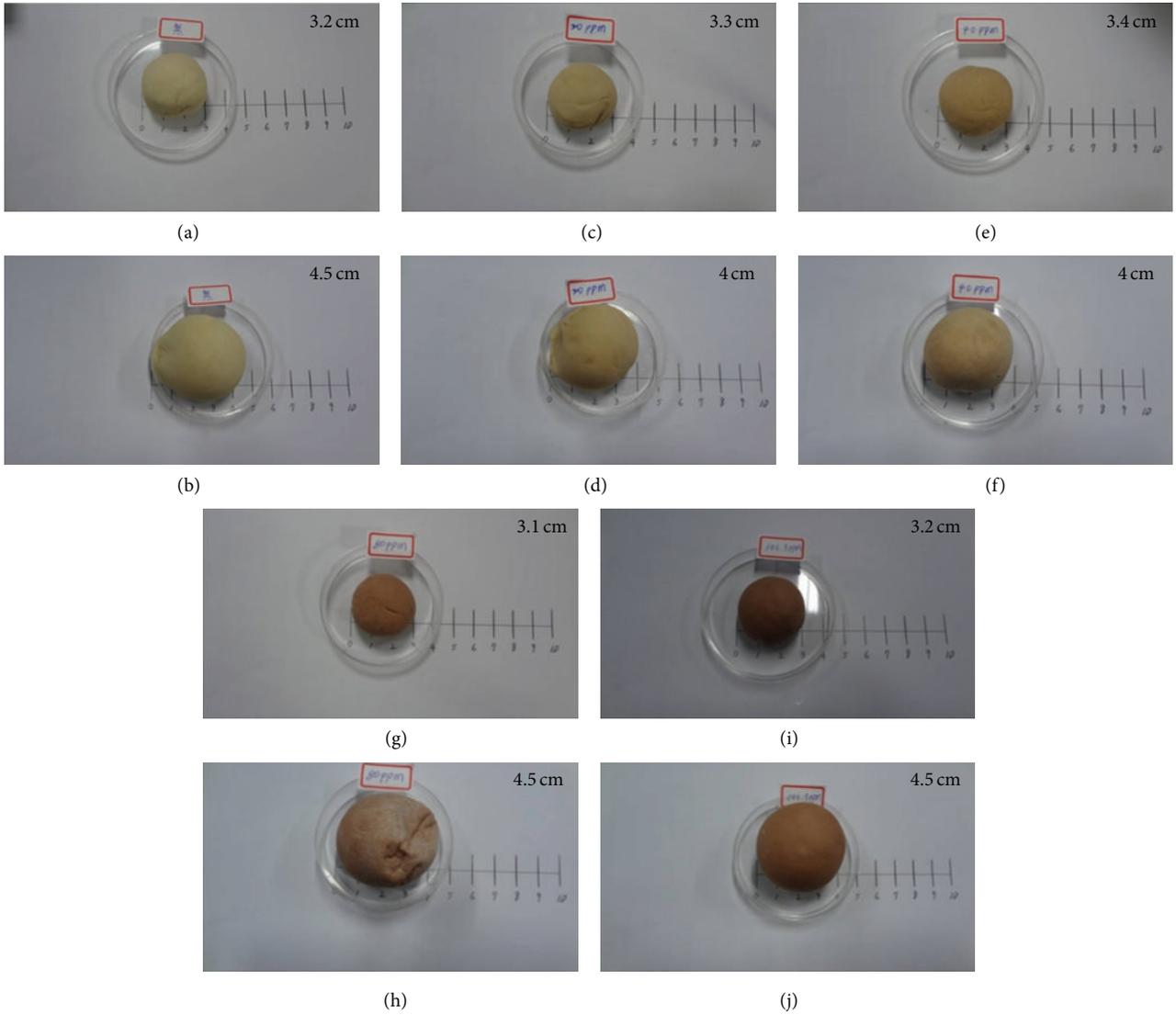


FIGURE 7: Experiment on antibioactivity of nanosilver for yeast.

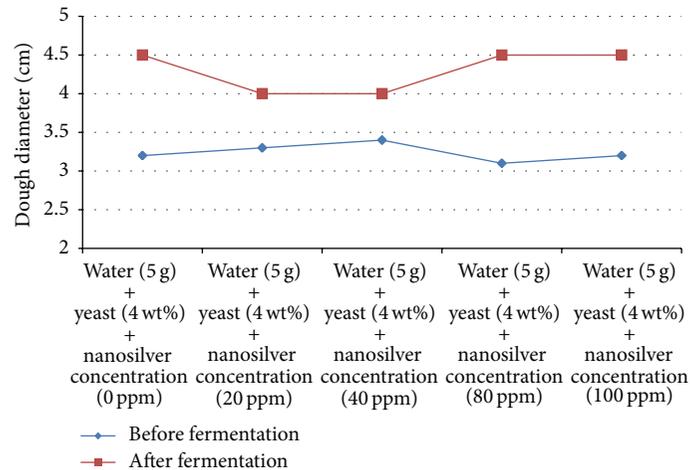
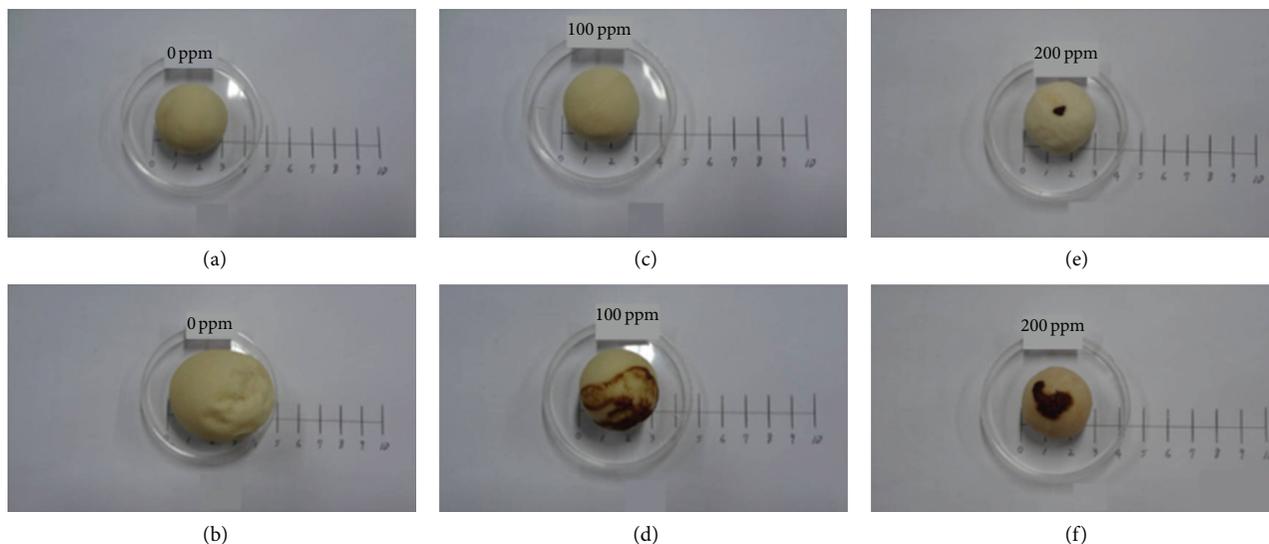
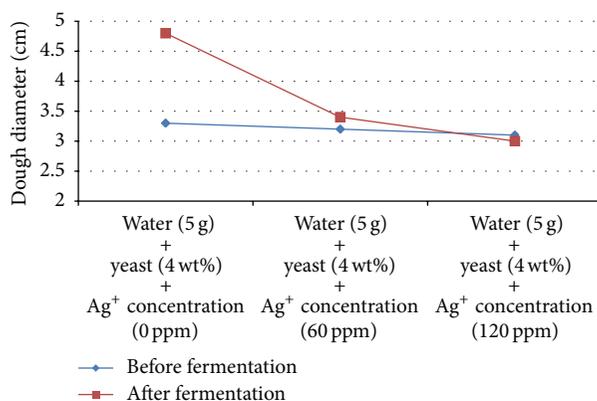


FIGURE 8: Experimental result of antibioactive effect of nanosilver.

FIGURE 9: Experiment on antibioactivity of  $\text{AgNO}_3$  for yeast.FIGURE 10: Experimental result of antibioactivity of  $\text{Ag}^+$ .

effective than silver nanoparticles at the same concentration (Figure 10). The possibility of dissociation capability of silver nanoparticles can be further explored to promote antibioactivity. In addition, *Staphylococcus aureus* had 2.8 million ~2.903 million base pairs and 2,600 genes, while *Escherichia coli* had 4.6 million base pairs and 4,000~5,500 genes. The yeast for fermentation had 120 million base pairs and 6,275 genes. Theoretically, the unicellular organisms with more genomes are more powerful to resist the  $\text{Ag}^+$ . Therefore, if nanosilver colloid can eradicate yeast, it can exterminate *Staphylococcus aureus* and *Escherichia coli* with less concentration.

#### 4. Conclusions

The colloidal silver solution was successfully prepared using ESD without any surfactants, which is different from the traditional chemical process with some toxic chemical agents that may influence the antibioactivity efficacy of colloidal

silver experiment. The properties of the self-made nanosilver colloid were analyzed and antibioactivity was tested and concluded, as follows.

- (1) According to the particle distribution analysis of water phase nanosilver, the particle size of nanosilver colloid was about 30~50 nm.
- (2) According to surface potential detection, the silver nanoparticle surface had  $-39.3$  mV voltage, and the surface potential was greater than 30 mV; thus, suspension stability was excellent. It is unlikely to deposit at normal temperature and pressure.
- (3) The silver nanofluid prepared by ESD had poor antibioactivity due to too low dissociation rate of  $\text{Ag}^0$ , and even if the concentration of nanosilver in the dough increased from 20 ppm to 100 ppm (the highest concentration in this study), it cannot fight against yeast.
- (4) The yeast used in this experiment could not resist 100 ppm  $\text{AgNO}_3$ , which is equivalent to 60 ppm silver ion. Previous literature proved that *Staphylococcus aureus* could not resist  $\text{Ag}^+$  by 20 ppm silver ion; thus, the resistance to silver ion of yeast is about 3 times that of *Staphylococcus aureus* due to the fact that assumption of genes number of the yeast is three times larger than the genes number of the *Staphylococcus aureus*.

#### Conflict of Interests

The authors declare that there is no conflict of interests regarding the publication of this paper.

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## Research Article

# Fabrication of Silver Interdigitated Electrode by a Stamp Method

**Kan-Sen Chou and Chia-Hsuan Lee**

*Department of Chemical Engineering, National Tsing Hua University, Hsinchu 30013, Taiwan*

Correspondence should be addressed to Kan-Sen Chou; [kschou@che.nthu.edu.tw](mailto:kschou@che.nthu.edu.tw)

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A stamp method was developed in this study to fabricate interdigitated electrodes (IDEs) on glass substrate from a 37.5 wt% silver ink. This method is simple and fast. A small amount of silver ink was first dripped into an IDE-patterned sponge of a stamp and then one could stamp out the desired IDE pattern made of nanosized silver colloids on a glass substrate, which was subsequently sintered at 280°C for 10 minutes to obtain the final silver IDE. Our brief study showed that when a large stamping force was used, more ink would be stamped out in the beginning and it decreased after each usage. However, if the force was too small, there would not be sufficient ink for a complete IDE. There existed therefore an optimal force to fabricate IDEs with minimal changes from sample to sample. The average dimension of an IDE when the applied force was 102 gm was roughly  $403 \pm 20 \mu\text{m}$  in width and  $1154 \pm 153 \text{ nm}$  in height, and the average final electrical resistivity was about  $10 \times 10^{-6} \Omega\text{-cm}$ .

## 1. Introduction

Interdigitated electrodes (IDEs) are widely used for various sensor applications, such as gas sensor, humidity sensor, biosensor, and so forth [1–10]. The IDE is often chosen as a component for those sensing operation, where electrical signals generated by the sensing material have to be detected via IDEs. In many of these examples, the IDE was fabricated by photolithography in a lift-off process. In some cases, very fine lines, in the range of 250–500 nm in width, can be produced in high density in this manner. The number of fingers can be up to 2000. More often, the width of the digit (finger) is on the order of 3 to 15  $\mu\text{m}$  and the number of fingers is around several hundred for this method. Several different metals had been used for this application, for example, Au, Pt, and Pd-Ag, which were often deposited by sputtering or e-beam evaporation and its thickness is in the range of 30 to 300 nm.

The photolithography process consists of many steps and requires the use of special equipment. Therefore, there are a few efforts in recent years to try to simplify the fabrication process for IDEs. For example, Kim et al. [11] used a laser-printing technology with silver ink on a glass substrate. By

adjusting printing parameters, the authors were able to produce IDEs having width between 6 and 28  $\mu\text{m}$  and thickness below 1  $\mu\text{m}$ . The resistivity of the resulting Ag IDE was  $\sim 20 \mu\Omega\text{cm}$ . Screen printing was another technique adopted to fabricate usable IDEs for various sensor applications [12, 13]. On the other hand, Tseng et al. [14] used ink jet printing to first print Pd colloids, followed by electroless plating of Ni on polyethylene terephthalate (PET) substrate. Successful patterns could be obtained when the line width was above 30  $\mu\text{m}$ . However, they have to perform a gold displacement process afterwards to improve the electrical conductivity of the interdigitated sensors.

The objective of this work is to develop a simple method to fabricate an IDE on glass substrate by using a stamp and silver ink. The concept of using a stamp to produce desirable pattern had been reported for several different systems [15–18]. The dimensions of final patterns depend on the methods for producing similar patterns in the stamp. Here, we will use a relatively fast and cheap method to produce the stamp to be used in association with silver ink to fabricate interdigitated electrodes. We will demonstrate the feasibility of this process and also report the dimensions of the resulting IDEs, which can be used for various sensor studies in the future.

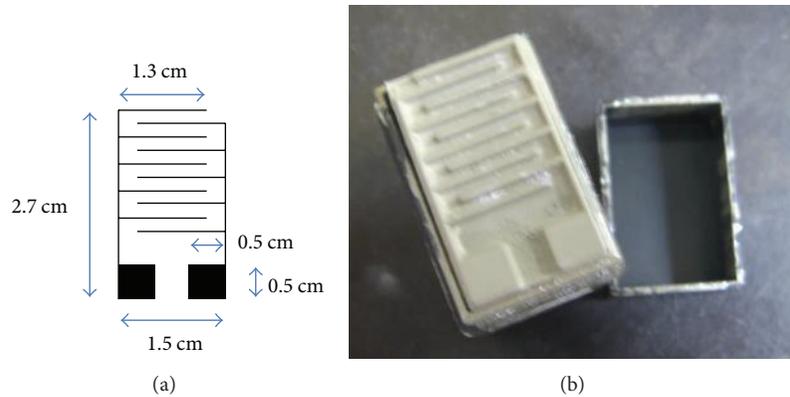


FIGURE 1: Schematic diagram of the dimensions of interdigitated electrode and the actual stamp with IDE pattern.

## 2. Materials and Methods

Shown in Figure 1 is a schematic diagram about the dimension and structure of the IDE (with five fingers) fabricated in this work. Spacing between fingers was designed at about 2 mm. However, in reality, after the ink was pushed out of the stamp, the silver colloids usually spread a little bit so that the spacing would be shortened to 1.3 to 1.5 mm.

This IDE pattern was first carved via hot metal plate into an ordinary stamp. To begin the experimental procedure, 0.15 mL of 37.5 wt% silver ink (Da-Shuan Applied Material, Hsinchu, Taiwan) (viscosity 6.3 centipoise) was uniformly dripped into the stamp. After about 10–15 minutes, when all the ink was completely soaked by the stamp, patterns of IDE could be stamped onto glass substrates (two IDEs on each substrate). About 40–50 patterns could be consecutively produced with this amount of ink. Two different forces, 102 g and 152 g, were tested in this study. These two forces were generated by simply glue several glass slides together. The stamp was pushed straight down with the above force for about 1 second to let ink flow onto the substrate before lifting. These samples were subsequently sintered at 280°C for 10 minutes to obtain the final IDEs.

$\alpha$ -step (Dektek 150, Veeco, USA) was used to measure the profiles (width and height) of the IDE fingers. Here, the width was measured in micron and height in nm. These measurements were repeated three times for each sample and averages were recorded. The surface morphology was observed by scanning electron microscopy (SEM, S-4700 I, Hitachi, Japan) and the volume resistivity determined from data by 4-point probe (2400-C SourceMeter, Keithley, USA) and  $\alpha$ -step measurements.

## 3. Results and Discussion

Shown in Figure 2 are the pictures of the sponge Figure 2(a), sponge loaded with silver ink Figure 2(b), and the final IDEs on glass substrate Figure 2(c). The color of the sponge changed somewhat after ink loading. When the stamp was pushed down by a suitable force, a certain amount of ink could flow out of the sponge onto the glass substrate to

form the desired pattern. The surface morphology after sintering was exhibited in Figure 2(d). The silver colloids were connected by the heat treatment to provide passage for electrical conduction. There might be some small sunken area (upper left region in the picture) probably caused by the lift action of the stamp. However, it is expected not to influence the electrical conductivity of the IDE.

**3.1. Effect of Applied Force.** Preliminary studies indicated that the amount of ink which was pushed out of the stamp was not enough to form a continuous pattern of IDE when the applied force was less than 100 gm. Results from two different forces, that is, 102 and 150 g, were presented here. First, shown in Figure 3 are examples of these profiles from  $\alpha$ -step measurement. These schematic profiles were not drawn in proportion. The units for width and height were in  $\mu\text{m}$  and nm, respectively. The maximum height, average height, and average width were shown for each profile. Average height was determined by dividing the total area under the profile by the average width, which was performed by the instrument itself. These data will be used for correlation with sample numbers.

Next, shown in Figure 4 are the correlations of average height and average width with sequence number of samples for the two cases of different applied forces. Clearly, in both cases, the width and height decreased with sequence number. However, the decreasing trend is smaller when the applied force was 102 gm. Using a larger force, more ink will flow out of the stamp in the beginning and decreased at a more rapid rate after each usage. As a result, both average height and width decreased more noticeably with usage as shown in these figures. Since the stamping action was manual, there were some inevitable fluctuations as expected. Nevertheless, they were within the range of expectations. When the applied force is 102 gm, we could not get conductive samples after number 20 due to insufficient ink being pushed out of the stamp. In view of these results, one can therefore in the future devise a scheme where the applied force is increased gradually, or stepwise, to minimize the differences between samples.

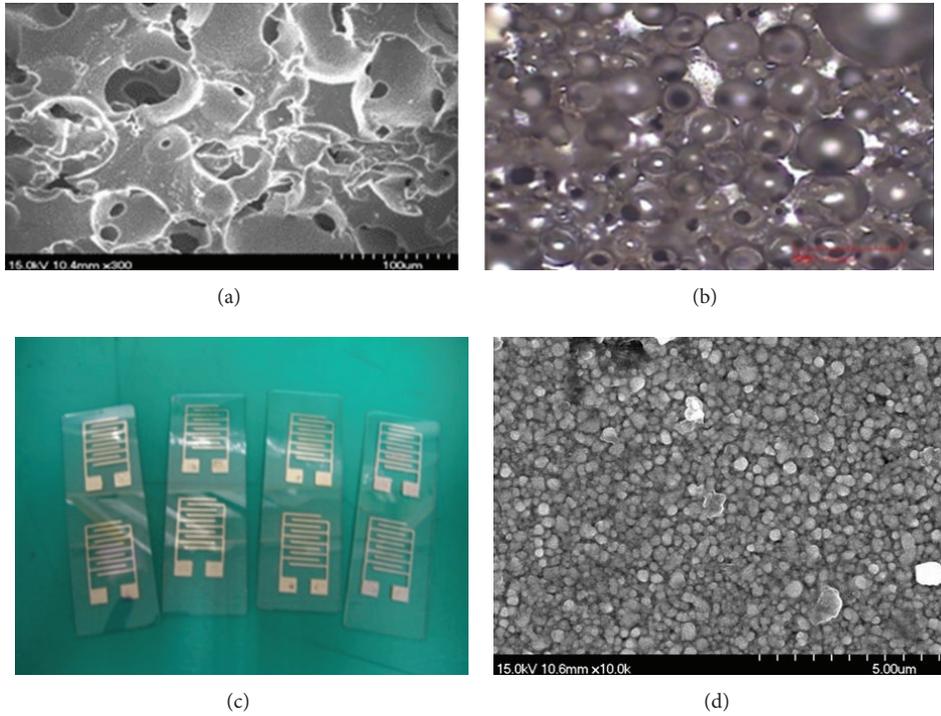


FIGURE 2: Photographs of (a) sponge of the stamp; (b) sponge loaded with silver ink; (c) final IDEs obtained in this work; (d) SEM of top surface of silver electrode after sintering.

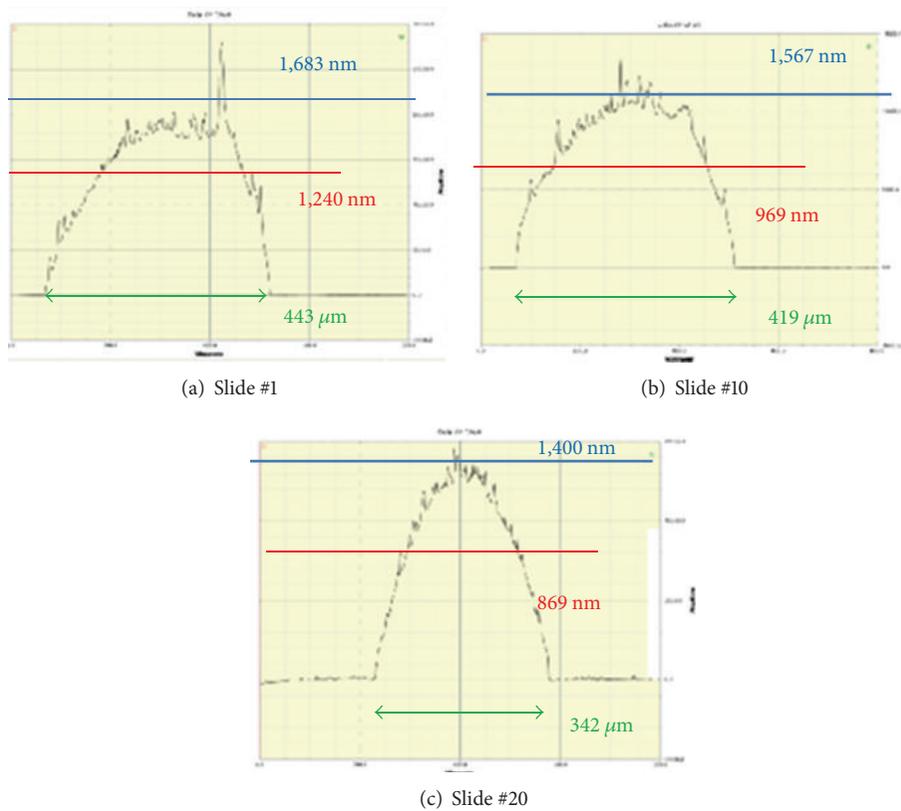


FIGURE 3: Representative results of profiles from  $\alpha$ -step measurement. top: max height, middle: average height, and bottom: average width.

TABLE 1: Volume resistivity of a few IDE samples.

Sample	1	2	3	4	5	6	7	8	9	10	Average
Volume resistivity $10^{-6} \Omega\text{-cm}$	13.5	13.4	17.3	19.4	5.3	3.2	8.3	2.4	7.3	8.0	$10.0 \pm 5.8$

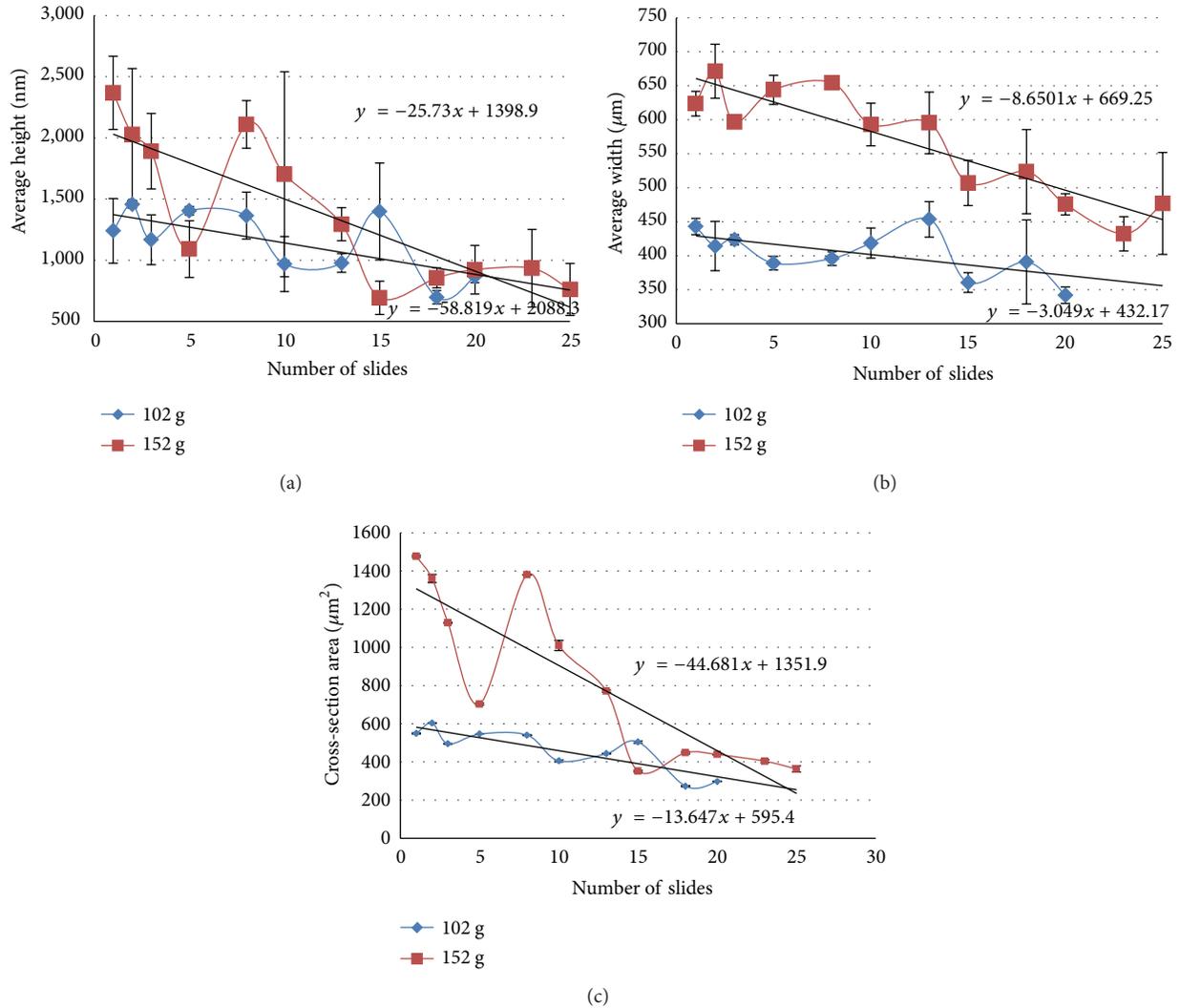


FIGURE 4: Results of the stamp method to fabricate IDE: (a) average height; (b) average width; and (c) cross-section area of finger in IDE versus the sequence number of slides.

Finally, the electrical resistivity was determined for a few samples and the data were exhibited in Table 1. Some degree of variations was observed, probably due to our manual operation in this study. Nevertheless, the average value at  $10 \times 10^{-6} \Omega\text{-cm}$  of the IDE seemed reasonable for many future potential applications.

#### 4. Summary

A simple stamp process was demonstrated to be feasible to quickly generate IDEs with reasonable electrical conductivity. All what were needed were silver ink and a patterned stamp. Our results indicated that when the applied force was 150 gm,

the amount of ink being pushed out of the stamp decreased more rapidly than the case when the force was only 102 gm (i.e., variation between samples being larger). The average dimension of an IDE when the applied force was 102 gm was roughly  $403 \pm 20 \mu\text{m}$  in width and  $1154 \pm 153 \text{ nm}$  in height, and the average electrical resistivity was about  $10 \times 10^{-6} \Omega\text{-cm}$ . These IDEs can be used in future sensor studies. In theory, the important parameters of this method include at least magnitude of applied force, duration of pressing, and dimension of patterns on the stamp. The dimension of IDEs fabricated by this method may be further decreased if one can generate finer IDE pattern on the stamp; increase ink viscosity by increasing solid content in the ink; and so forth. Moreover, the variation between samples can be minimized

if the operation can be performed automatically instead of manually. More studies are obviously needed to understand more details about this new and simple technique.

### Conflict of Interests

The authors declare that there is no conflict of interests regarding the publication of this paper.

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