Synthesis of Novel Photomediated Silver Nanoprisms via a Light-induced Transformation Reaction for Use as Anti-bacterial Wound Dressing

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ABSTRACT

Silver nanoprisms are useful for medical applications as an antimicrobial agent due to their large surface area hence providing better contact with microorganisms. Yet, they are toxic to mammalian cells. To alleviate this problem, synthesis of silver nanoprisms via the light-induced transformation reaction with 530±20 nm LED light is purposed. Under the proper conditions, this reaction is controllable and exceptionally efficient with high transformation yields. Additionally, it is eco-friendly which excluded the hazards from the use of harmful oxidizing agents. The synthesized photomediated silver nanoprisms (PAgNPrs) were characterized by Transmission electron microscopy (TEM), X-ray Diffractometer (XRD) and UV-visible spectroscopy. The antibacterial effect of the PAgNPrs against Escherichia coli, Pseudomonas aeruginasa, Enterococcus faecalis and Methicillin-resistant Staphylococcus aureus (MRSA) by determining both MIC and MBC is reported. The results obtained from various characterizations revealed that PAgNPrs were in spherical, decahedron, triangular prism and truncated octahedron shape. The MIC and MBC of the synthesized photomediated silver triangular nanoprisms was found to be optimum at 2.5 mM which is considered very low when compare to the commercial silver-containing dressing in the market. Moreover, the synergistic activity of the PAgNPrs with bacterial celluloses (BC) against above mentioned bacteria have better antibacterial effect as compare with pure bacterial cellulose. Thus, the photomediated silver triangular nanoprisms can be used as the remarkably effective antibiotics wound dressings.

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INTRODUCTION

Silver metals have been used for centuries by humans in different applications as construction materials, coins, weapons, decorations, etc. Traditionally, the use of silver powder as wound healing and treatment of ulcers is widespread. In medicine, silver plays a crucial role as an antimicrobial agent in different biomedical fields, especially in treatment of burns. However, silver metals are not only toxic to microorganisms, but also mammalian cells. In contrast to bactericide effects of silver metals, the antimicrobial activities of silver metals are manipulated by the sizes and dimensions of the particles. As the nanoscale materials, they have come out as novel antimicrobial agents due to their high surface area to volume ratio. Importantly, the prism shape of materials have shown efficient antimicrobial efficacy owing to their large surface area hence providing better contact with microorganisms. Thus, synthesis of nontoxic antibacterial silver nanoprisms with broad-spectrum of anti-bacterial activity is purposed.
In developing routes of synthesis, several studies have made to control the size and shape of silver nanoparticles. Silver nanoparticles have been produced using different methods such as reduction of metal salts, electrochemical method, thermal decomposition, laser ablation or microwave irradiation. The synthesis of silver nanoprism under light was first reported by C. Mirkin et al in 2007\(^4\), they synthesized silver nanotriangles using fluorescent light. Herein, we report a new method for the synthesis of silver nanoprism through the light-induced transformation of the spherical silver nanoparticles into photomediated silver nanoprism (P\(_{\text{AgNPrs}}\)) via excitation with 530±20 nm LED lights. This reaction is both synthetically useful and mechanistically intriguing\(^4\)-\(^7\). LED light is used as the fundamental medium to convert the seed particles into nanoprism, and control their shape and optical properties. It allows one to synthesize silver nanoprism with relatively narrow plasmon bands that span the visible and NIR regions of the spectrum\(^4\). The important part of the synthesis via this reaction is that it can be turned on and off by just switching on and switching off the light source and observed the reaction through the color change of the solution by naked-eyes. Moreover, this reaction is ecofriendly method which excluded the hazards from the use of harmful oxidizing agents.

Though, there are several studies of MIC and MBC of silver nanoparticles on bacteria has been reported. Investigation of the antibacterial effect of the photomediated silver nanoprism (P\(_{\text{AgNPrs}}\)) on both gram positive and negative bacteria has not been established yet. The aim of this study is to assess the antimicrobial efficacy of P\(_{\text{AgNPrs}}\) against \textit{Escherichia coli}, \textit{Pseudomonas aeruginosa}, \textit{Enterococcus faecalis} and \textit{Methicillin-resistant Staphylococcus aureus (MRSA)} by determining both MIC and MBC.

To further develop the effective wound dressings, bacterial cellulose (BC) is a new proposition, which is being constantly used. BC is a purified form of extracellular polysaccharide composed of long non-aggregated nanofibrils that is produced by Gram-negative bacteria using glucose\(^8\). BC has a wide range of potential applications in many fields such as a separation membrane, a mixing agent, a viscosity modifier in food industry, membranes for fuel cells, fibers in paper industrial, an artificial skin, dental implants, vascular grafts, dialysis membranes, membrane in antibacterial applications, controlled-drug release carries, tissue replacement, etc. Interestingly, BC was found to be a promising material for wound dressing due to its fibril network structure, which is very similar to the extracellular matrix of human skin, and its outstanding holding water capacity, ultra-fine network structure, high tensile strength, high crystallinity, high purity, hydrophilicity, biocompatibility and degradability\(^9\)-\(^13\). According to the above mentioned, BC, undoubtedly, is another interesting and very attractive alternative choice of wound care. However, the major drawback of applying BC as a wound dressing is its poor antimicrobial activity, which means that it cannot guarantee that the wound infection would not appear. Addition of some substances with specific properties into BC can improve its properties\(^14\)-\(^15\). Thus, our group decided to incorporate the optimal synthesized photomediated silver nanoprism into BC medium in order to be a promising material for wound dressing.

**EXPERIMENTAL**

\textbf{A. Chemicals and materials}

Silver nitrate (Analysis EMSURE ACS, ISO, Reag. Ph Eur) was purchased from Merck Milipore. Tri-sodium citrate dehydrate (99.0\%) was purchased from Ajex Finechem. Sodium borohydride (\(>96\%) and Bis(p-sulfonatophenyl)phenylphosphate dehydrate dipotassium (BSPP, 97\%) were purchased from Aldrich and used as received. All \(\text{H}_2\text{O}\) was
purified by a Barnstead Nanopure water purification system (resistance = 18.1 MΩ). The stock solutions were prepared fresh and put on ice before using. All the vials are cleaned with acetone and the sodium borohydride solution is purged with nitrogen before actually adding the borohydride salt.

B. Bacteria strains, medium, and cultivation

Escherichia coli reference strain (ATCC–8739), Pseudomonas aeruginosa reference strain (ATCC–27853), Enterococcus faecalis reference strain (ATCC–19433) and Methicillin-resistant Staphylococcus aureus (MRSA) from faculty of medicine (Chulalongkorn University, Thailand) was revived under sterile condition. Then the concentration of the bacteria was adjusted by diluting in 0.85% saline to an optical density of 0.10 at 625 nm (1x10^8 CFU/ml, 0.5 McFarland’s standard).

C. Preparation of Silver Nanoparticles

Nanopure water (95 mL), AgNO₃ (0.5 mL, 50 mM), and sodium citrate (1 mL, 30 mM) were combined in a 250 mL three-neck flask. The flask was immersed in an ice bath, and the solution was bubbled with N₂ under vigorous stirring for 2 hours. One milliliter of aqueous NaBH₄ (50 mM, freshly prepared with ice-cold Nanopure water prior to injection) was rapidly injected into the solution. After adding aqueous NaBH₄, stop purging the solution. Over the next 5 minutes, 100 µL of NaBH₄ solution were added every 2 minutes into the solution (at 7, 9 and 11 minutes). Then 1 mL of BSPP (5 mM) and 500 µL of NaBH₄ solutions were mix together and put on the ice. After 15 minutes, 150 µL of this mix solution was added to the reaction mixture and repeats this step every 30 seconds until all of the mix solution is gone. The resulting Ag colloid was gently stirred for at least 15 hours in the ice bath and allowed to age overnight at around 4 °C in the dark.

D. Photomediated Silver Nanoprism Growth

The starting pH of the aged silver nanoparticle solution will be around 9.0. To achieve photo-controlled unimodel growth, aqueous sodium hydroxide (0.2 M NaOH) was introduced dropwise into a solution while shaking to elevate the pH to 9.5, 10.0 and 11.0. After the pH conversion, the solution was pour into small vials (20 mL) which have been cleaned with acetone. A solution of silver nanoparticles, at a pH of 9.5, 10.0 and 11.0, then were irradiated under a 2-Watt LED with wavelength 530±20 nm. The solution was put under directly as much of the LED as possible, and run the LED at about its maximum current. Then cover with a black cloth for 0-120 hours.

RESULTS AND DISCUSSION

A. Photoconversion of silver nanospheres into nanoprisms under LED light

Synthesis of the PAgNPrs in aqueous solution was monitored by recording the absorption spectra at a wavelength range of 300-900 nm. The initial pH of the silver nanoparticle solution prior to irradiation was determined to be 9.5 with a benchtop pH meter. The physical appearance of the silver nanoparticles solution was yellow. Detailed studies of these by UV-Visible spectroscopy show the characteristic plasmon band signature at 399 nm associated with the spherical particles. A typical photoreaction was carried out by irradiating the silver nanoparticle solution with 530±20 nm light using 2-Watt LED lamps as the light source. After 8 hours of irradiation, the physical appearance of these silver nanosphere solution turned from yellow into green color and the characteristic
plasmon band signature at 399 nm exhibited a decrease in intensity of which gradually emerged a new band at 614 nm. These results indicated that the silver nanospheres were converted to nanoprisms. In every next 2 hours, the physical appearance of the solution turned into darker shades of green color and new bands started to shift consistently to the right side which indicated to the growth of silver nanoprisms size. As the silver nanoprisms are protected by negatively charged ligands, tri-sodium citrate salt and bis(p-sulfonatophenyl)phenylphosphine (BSPP), the repulsion between the silver nanoprisms in solution will be dependent on the net negative charge on the silver nanoprisms surface (SCHEME 1). Irradiation with LED light can induce optical attractive forces between the silver nanoparticles leading to enhance at dipole plasmon resonance wavelengths as the plasmon bands of the prisms are red-shifted with respect to the excitation time. However, after irradiation for 36 hours, the physical appearance of the solution turned from green to blue while new bands started to shift consistently to the left side. As the silver nanoprisms are strongly excited by the LED light during the growth process, the tips and edges are polarized to the extent that they can overcome electrostatic repulsion and bring two or more nanoprisms together and stops. When the prism no longer absorbs the excitation wavelength, the intensity band of spherical particles disappears and the silver nanospheres will be completely converted to nanoprisms (FIGURE 1B). For the initial pH 10.0 of the silver nanoparticle solution, the change in physical appearances and UV-visible spectroscopy of the reaction is mostly similar to pH 9.5; however, the change in color is faster comparing to the same excitation time (FIGURE 1A). Unlike the others, the physical appearance of the initial pH 11.0 of the silver nanoparticle solution showed no sign of blue color shades (FIGURE 1A).

Transmission electron microscopy (TEM) provided insight into the morphology and size details of the synthesized PAgNPrs. When the silver nanospheres are excited by LED light of single wavelength 530±20 nm converting to the solution of silver nanoprisms with various shapes, they can be categorized into 4 morphologies: spherical, decahedron, triangular prisms and truncated octahedrons. The TEM images at different magnifications are depicted in the (FIGURE 4). The spherical shape of the PAgNPrs was determined by the TEM with average diameter ranging 10-20 nm (FIGURE 4A and E). The decahedron of the PAgNPrs have average diameter around 130 nm (FIGURE 4B and F). The triangular prisms of the PAgNPrs was determined by the TEM also with average diameter ranging 60-70 nm (FIGURE 4C and G) and ranging 160-170 nm for the truncated octahedrons of the PAgNPrs (FIGURE 4D and H).

SCHEME 1 Proposed diagram of the reaction occurring on the formation of various shapes of photomediated silver nanoprisms.
FIGURE 1 A) The photographs and B) corresponding extinction spectra of the photomediated silver nanoprisms solution at pH 9.5, 10.0 and 11.0 with 530±20 nm LED irradiation and difference irradiation time respectively.

FIGURE 4 (A) TEM image of silver nanospheres at 50 nm range (B) TEM image of the photomediated silver nanodecahedron at 100 nm range (C) TEM image of the photomediated silver nanotriangular prisms at 200 nm range (D) TEM image of the photomediated silver nanotruncated octahedrons at 200 nm range (E) TEM image of silver nanospheres at 20 nm range (F) TEM image of the photomediated silver nanodecahedron at 50 nm range (G) TEM image of the photomediated silver nanotriangular prisms at 50 nm range (H) TEM image of the photomediated silver nanotruncated octahedrons at 100 nm range.

B. Antibacterial activity of the synthesized photomediated silver nanoprisms
The antibacterial efficacy of the synthesized PAgNPrs was determined by the value of MIC and MBC via examination using standard broth dilution method against *Escherichia coli*, *Pseudomonas aeruginosa*, *Enterococcus faecalis* and *Methicillin-resistant Staphylococcus aureus* (MRSA). In this step, the photomediated silver nanoparticles in spherical, decahedron, triangular prism and truncated octahedron shape were tested respectively.

*Escherichia coli*; After 24 hours of incubation under aerobic condition at 37˚C, turbidity was noticed in all the eppendorfs ranging from concentrations of 0.01563 to 0.312 mM containing the synthesized photomediated silver nanoprisms (PAgNPrs), indicating growth of bacteria. Whereas in concentrations of 0.625, 1.25 and 2.5 mM, no turbidity was seen exhibiting inhibition of bacterial growth. The suspension from the all eppendorfs were inoculated in Mueller Hinton agar plate and incubated for 24 hours and no growth of bacteria was observed in the concentrations of 0.625, 1.25 and 2.5 mM respectively hence confirming it bactericidal. Therefore, the optimum MIC was obtained with 0.625 mM. These results thus confirm that the MIC and MBC of the synthesized PAgNPrs were found to be effective at dilution of 0.6255 mM.

*Pseudomonas aeruginosa*; After 24 hours of incubation under aerobic condition at 37˚C, turbidity was noticed in all the eppendorfs ranging from concentrations of 0.01563 to 1.25 mM containing photomediated silver spherical and truncated octahedron nanoprisms indicating growth of bacteria. Whereas in the eppendorfs containing photomediated silver decahedron and triangular nanoprisms, turbidity was noticed in the eppendorfs ranging from 0.01563 to 0.625 mM. However, in concentrations of 2.5 mM of photomediated silver spherical and truncated octahedron nanoprisms and in concentrations of 1.25 mM of photomediated silver decahedron and triangular nanoprisms, no turbidity was seen exhibiting inhibition of bacterial growth. The suspension from all the eppendorfs were inoculated in Mueller Hinton agar plate and incubated for 24 hours resulting in no growth of bacteria was observed in the concentrations of 1.25 mM for the photomediated silver spherical and truncated octahedron nanoprisms and 1.25 and 2.5 mM for the photomediated silver decahedron and triangular nanoprisms hence confirming it bactericidal. These results thus confirm that the MIC and MBC of the photomediated silver spherical and truncated octahedron nanoprisms were found to be effective at dilution of 2.5 mM and the MIC and MBC of the photomediated silver decahedron and triangular nanoprisms were found to be effective at dilution of 1.25 mM.

*Enterococcus faecalis*; After 24 hours of incubation under aerobic condition at 37˚C, turbidity was noticed in all the eppendorfs ranging from concentration of 0.01563 to 2.5 mM containing the photomediated silver spherical and decahedron nanoprisms indicating growth of bacteria. Whereas in the eppendorfs containing the photomediated silver triangular and truncated octahedron nanoprisms, turbidity was noticed in the eppendorfs ranging from 0.01563 to 1.25 mM. However, in concentrations of 2.5 mM of the photomediated silver triangular and truncated octahedron nanoprisms, no turbidity was seen exhibiting inhibition of bacterial growth. The suspension from all the eppendorfs were inoculated in Mueller Hinton agar plate and incubated for 24 hours resulting in no growth of bacteria was observed in the concentrations of 2.5 mM for the photomediated silver triangular and truncated octahedron nanoprisms hence confirming it bactericidal. These results thus confirm that the MIC and MBC of sample the photomediated silver triangular and truncated octahedron nanoprisms were found to be effective at dilution of 2.5 mM.
whereas the photomediated silver spherical and decahedron nanoprisms are probiotics against *E. faecalis*.

*Methicillin-resistant Staphylococcus aureus (MRSA)*: After 24 hours of incubation under aerobic condition at 37°C, turbidity was noticed in all the eppendorfs ranging from concentration of 0.01563 to 1.25 mM containing the photomediated silver spherical, decahedron and triangular nanoprisms indicating growth of bacteria. Whereas in the eppendorfs containing the photomediated silve truncated octahedron nanoprisms, turbidity was noticed in the eppendorfs ranging from 0.01563 to 2.5 mM. However, in concentrations of 2.5 mM of the photomediated silver spherical, decahedron and triangular nanoprisms, no turbidity was seen exhibiting inhibition of bacterial growth. The suspension from all the eppendorfs were inoculated in Mueller Hinton agar plate and incubated for 24 hours resulting in no growth of bacteria was observed in the concentrations of 2.5 mM for the photomediated silver spherical, decahedron and triangular nanoprisms hence confirming it bactericidal. These results thus confirm that the MIC and MBC of the photomediated silver spherical, decahedron and triangular nanoprisms were found to be effective at dilution of 2.5 mM whereas the photomediated silver truncated octahedron nanoprisms is probiotics against *E. faecalis*.

**CONCLUSIONS**

In this study, novel approach for synthesis of photomediated silver nanoprismoids (PAgNPrs) via light-induce transformation reaction was given. The kinetic of the reaction was determined by UV-visible spectroscopy. The synthesized PAgNPrs were spherical, decahedron, triangular prisms and truncated octahedron with size raging around 10-20, 130, 60-70 and 160-170 nm respectively as observed in TEM. The synthesized PAgNPrs was aim to evaluated the antibacterial effect of *Escherichia coli*, *Pseudomanas aeruginasa*, *Enterococcus faecalis* and *Methicillin-resistant Staphylococcus aureus (MRSA)* by MIC and MBC. The Photomediated triangular silver nanoprisms displayed the strongest biocidal action, compared with spherical, decahedron and truncated octahedron nanoprisms. The MIC and MBC of photomediated triangular silver nanoprisms was found to be optimum at 2.5 mM which is considered relatively low comparing to the commercial silver-containing dressing in the market. Moreover, the synergistic activity of the photomediated triangular silver nanoprisms with bacterial celluloses (BC) against the bacteria above mentioned established their application as an effective wound dressing. Thus, it is concluded that synthesized PAgNPrs via light-induced transformation reaction with 530±20 nm LED light is a cost effective, and able to prevent the growth of bacteria effectively which can be incorporated with bacterial cellulose and developed as remarkably effective wound dressings.

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