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Green Synthesis of Anti-bacterial Nano Silver by Polysaccharide from Bletilla Striata

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Abstract: The silver nanoparticle is a good antibacterial material being used as a broad-spectrum fungicide, including against some multidrug-resistant strains. Compared with the normal chemical and physical preparation methods, green synthesis has attracted wide attention, because of the pharmaceutical activities of the natural product, mild reaction conditions, and environmentally friendly, etc. In this study, the synthesis of silver nanoparticles (Ag NPs) was prepared from *Bletilla striata* polysaccharide (BSP) and characterized by UV-vis spectroscopy and Dynamic Light Scattering (DLS). Scanning electron microscopy (SEM) and transmission electron microscopy (TEM) indicated the morphology of Ag NPs was subspherical with an average size of 20–35 nm. *Bletilla striata* polysaccharide not only can be used as a natural reducing agent, but also has good repairing ability. Moreover, the antibacterial experimental results showed its great antimicrobial activity against Gram-positive bacteria (*Staphylococcus aureus*), Gram-negative bacteria (*Escherichia coli*) and *Candida albicans*.

Keywords: silver nanoparticles; green synthesis; *Bletilla striata* polysaccharide; antibacterial

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1. Introduction

Silver nanoparticles (Ag NPs) are metallic silver nano particles in the range of 1–100 nm. The broad-spectrum antimicrobial of silver encourages its use in biomedical applications, water and air purification, food production, clothing, and numerous household products [1–3], and silver has a greater antimicrobial activity at the nano size [4,5]. There is a great interest in Ag NPs for medical applications compared with the bacterial resistance to other antibiotics. So far, there are no conclusive reports on the development of bacterial resistance to Ag NPs [6].

The most common methods to synthesize Ag NPs are chemical reduction and physical method [3,7]. For example, using strong reducing agents (borohydride or hydrazine) reduce the silver nitrate solution, laser ablation method or ball milling method to prepare Ag NPs. However, those traditional approaches involve the use of hazardous solvents, chemicals, toxic reagents, power-wasting equipment and poor size control [8–10]. Silver nanoparticles were considered bio-compatible, but chemical synthesis methods may still lead to the toxic chemical species absorbed on the surface [11]. Therefore, researchers have been persistently searching for more economical, environmentally friendly and safe Ag NPs synthesis with natural products. Green synthesis seems to be a potential strategy.

Green synthesis is using naturally occurring reagents such as plant extracts, vitamins, sugars, biodegradable polymers, and microorganisms as reductants and capping agents [9,11–13]. Making use of naturally occurring reagents to synthesize Ag NPs is an economic, environmental and safe method. Nowadays, many plant extracts and microorganisms are used to synthesize silver nanoparticles including bacteria [14–17], fungi [18–20], algae and plant extracts [4,7–9,21–27]. In addition, the reduction effect of plant extracts

from different parts of the preparation of silver nanoparticles is also one of the research focuses [28]. Plants are more preferable than microbes as agents for the synthesis of silver nanoparticles because they are readily accessible, and the purification process is simpler [29]. Polysaccharides are often used in green synthesis due to their wide source and strong reducing properties.

Bletilla striata polysaccharide (BSP) is a kind of high-viscosity glucomannan extracted from *Bletilla striata* tuber by water extraction and alcohol precipitation. It was usually formed by polymerization of α-mannose, β-mannose, and β-glucose [30,31]. BSP can participate in intracellular or intracellular cell signal transduction, showing a typical ability to enhance immune activity. It was published that the addition of BSP increased the ability to pierce biofilm and promoted wound healing [32]. Although BSP is widely used in biomedical applications, its application to Ag NPs synthesis has not been researched.

We reported herein a green, efficient and safe method for the preparation of Ag NPs-BSP using BSP as a reducing and stabilizing agent (Figure 1). In order to make Ag NPs-BSP more convenient to use, we prepared Ag gel and confirmed that the nanoparticles as well as its nanogel are good candidates for further biological applications. Additionally, the antibacterial ability of the Ag NPs-BSP and its nanogel prepared by our method was verified in *Staphylococcus aureus*, *Escherichia coli*, and *Candida albicans*, respectively. We expect that this green synthesis method could provide more ideas for the preparation of silver nanoparticles.

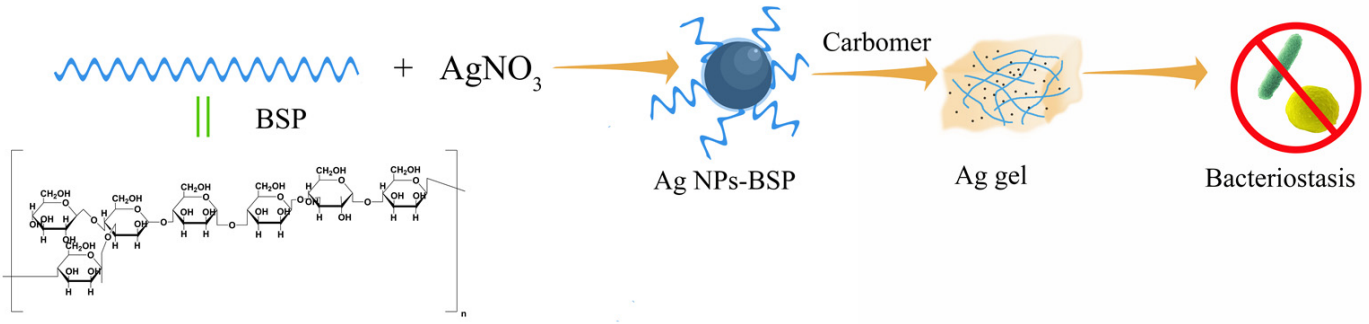

Figure 1. Schematic illustration of Ag NPs-BSP synthesis and verify antibacterial.

2. Results

2.1. Synthesis and Characterization

The color change from pale yellow to dark brown in response to time can be seen as evidence of silver ion reduced to Ag NPs. Ag NPs have apparent UV absorption because of surface plasmon resonance (SPR) characteristics. Scanned in UV, the sharp peak at around 435 nm evidenced the formation of silver nanoparticles (Figure 2a). Dynamic Light Scattering (DLS) was used to test size and zeta potential. The zeta potential of Ag NPs synthesized by BSP was −7.76 mV due to the residue of the BSP on the surface. After being washed, the zeta potential was decreased to −23.1 mV for the removal of BSP (Figure 2b). The lattice fringes with d = 0.203 and 0.238 nm in the high-resolution image of Ag NPs are (200) and (111) planes of Ag (Figure 2c), which are coinciding with the typical lattice. TEM image of Ag NPs was shown in Figure 2d. The particles are predominantly spherical with a diameter ranging from 20 to 35 nm. Detection by Inductively Coupled Plasma Spectrometer (ICP), the silver content was 43.5% in Ag NPs-BSP.

The elemental composition of the synthesized Ag NPs-BSP was assessed using EDX spectroscopy. There are strong silver peaks approximately at 3 keV, which is typical for the absorption of Ag (Figure 3a) [15]. Carbon and oxygen are evenly distributed on Ag NPs, shown in Figure 3b–e. There was organic matter on the surface of Ag NPs, which is due to the BSP attached to the surface.

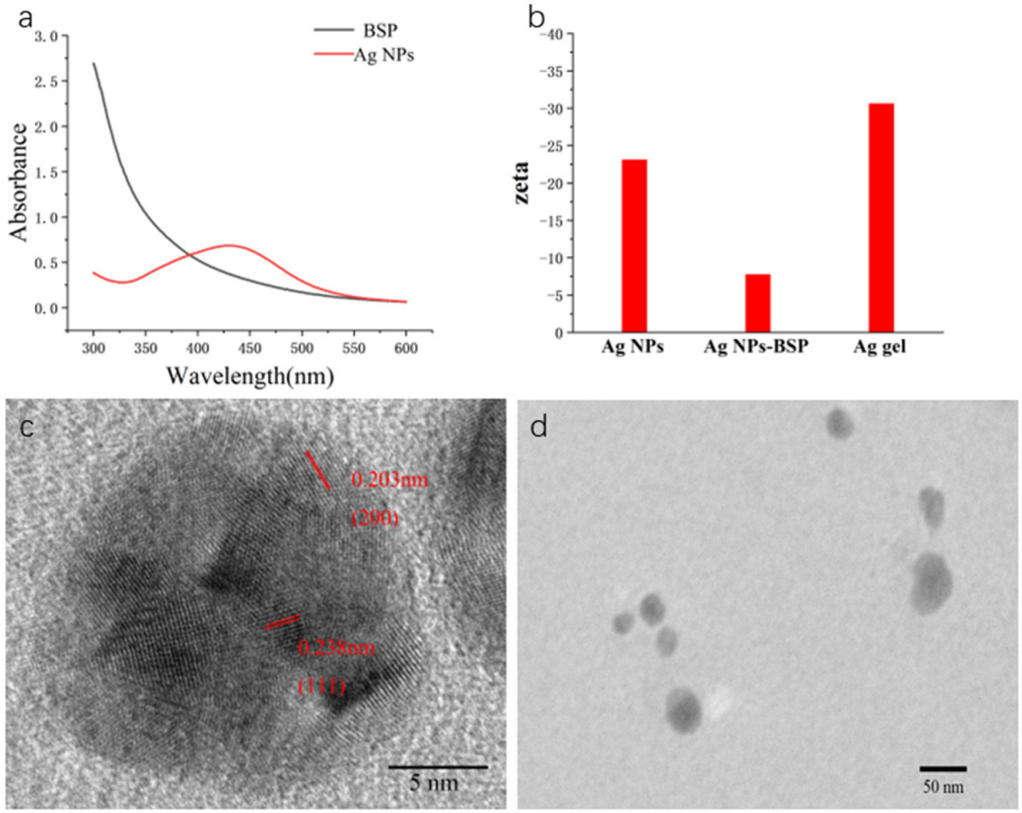

Figure 2. (**a**) UV–vis absorption spectra of Ag nanoparticles and *Bletilla striata* polysaccharide (BSP). (**b**) the zeta potential of Ag nanoparticles washed (named Ag NPs), Ag nanoparticles synthesized by BSP (named Ag NPs-BSP) and Ag nanoparticles carbomer gel (named Ag gel). (**c**,**d**) TEM and high-resolution image of Ag NPs.

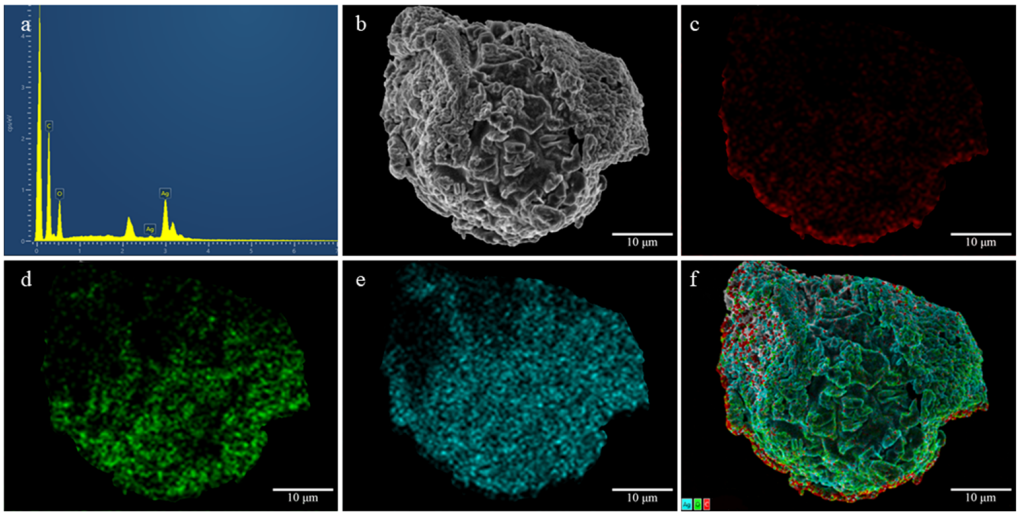

Figure 3. (**a**) EDX energy spectrum analysis, (**b**) SEM mapping of Ag NPs-BSP including (**c**) Carbon, (**d**) Oxygen, (**e**) Ag, and (**f**) merge.

2.2. Antibacterial Ability of Nano Silver

In order to verify the antibacterial activity of Ag NPs, measuring antibacterial activity of Ag NPs was evaluated by the pour plate method against *Escherichia coli* (ATCC 25922), *Staphylococcus aureus* (ATCC 25923), and *Candida albicans* (ATCC 14053). The Bacteriostatic rate of Ag NPs against *Escherichia coli*, *Staphylococcus aureus* and *Candida albicans* were 95.23%, 93.47%, and 98.40% after 5 min sterilization experiment. Similar results were

obtained with Ag gel antibacterial activity, 91.03%, 95.33% and 93.43% against *Escherichia coli*, *Staphylococcus aureus* and *Candida albicans* were observed. Ag NPs and Ag gel showed no significant difference ($p > 0.05$) in antibacterial activity against the three bacteria. The results showed that the antibacterial activity of silver nanoparticles was not decreased by the preparation of silver nanoparticles into gel. The results of the antibacterial activity showed that the synthesized Ag NPs had efficient antibacterial activity against both Gram-positive bacteria (*Staphylococcus aureus*), Gram-negative bacteria (*Escherichia coli*) and *Candida albicans* (Figure 4). The bacteriostasis rate is over 95%. There are several hypotheses explaining the antibacterial activity of nano silver: (1) generation of reactive oxygen species; (2) Ag+ ions from Ag NPs denaturize proteins by bonding with sulfhydryl groups; (3) attachment of Ag NPs on bacteria and subsequent damage to bacteria [5]. The great antimicrobial activity proved that the silver nanoparticles can be used as broad-spectrum fungicide.

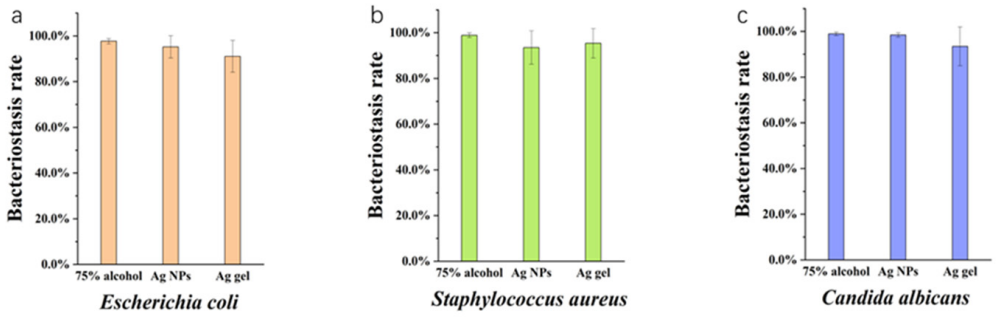

Figure 4. Antibacterial rate of 75% alcohol, Ag NPs (200 ppm) and Ag gel against (**a**) *Escherichia coli* (ATCC 25922), (**b**) *Staphylococcus aureus* (ATCC 25923) and (**c**) *Candida albicans* (ATCC 14053) (n = 3).

2.3. The Ability of BSP Promote Cell Migration In Vitro

To study the effect of BSP on the migration of fibroblasts, a scratch experiment was performed. BSP group was 110 μg/mL BSP (content of Ag NPs-BSP at 200 ppm) dissolved by low serum medium. After 48 h, NIH/3T3 cells in the BSP group migrated to the scratched area more than those in the control group (Figure 5). This proved that BSP can enhance and promote cell migration and wound healing in vitro.

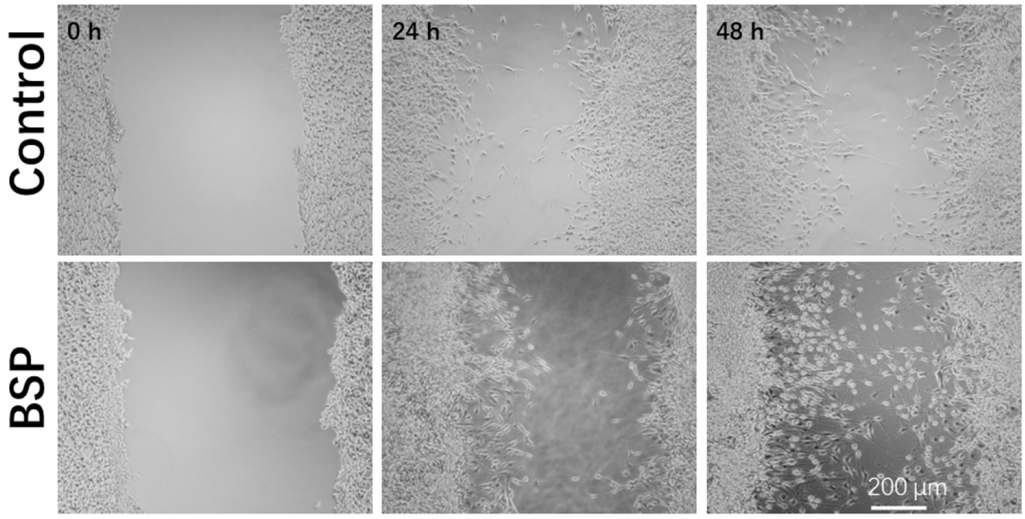

Figure 5. Images of wound scratch migration assay by the cell migration experiment.

3. Materials and Methods

3.1. Materials

Silver nitrate ($AgNO_3$) analytical grade, glycerol and propylene glycol were purchased from Sinopharm Chemical Reagent Co., Ltd. Shanghai China. BSP was bought from Shanghai yuanye Bio-Technology Co., Ltd. Shanghai China. Carbomer was obtained from

Shanghai Aladdin Bio-Chem Technology Co., Ltd. Shanghai China. Triethanolamine were purchased from Nanjing Chemical Reagent Co., Ltd. Nanjing China. All the chemicals were used as received without further treatment. Deionized water was used for all experiments. *Escherichia coli* (ATCC 25922), *Staphylococcus aureus* (ATCC 25923), *Candida albicans* (ATCC 14053) are from National Teaching Experimental Center, College of Life Science and Technology, China Pharmaceutical University Nanjing China.

3.2. Instrument

UV-Visible spectroscopy (UV) UV-3600 Plus (Shimadzu) Japan. Zetasizer Nano ZS90 (Malvern) Britain. Transmission Electron Microscope (TEM) HT7700 (HITACHI) Japan and high-resolution TEM JEM 2100 F (JEOL) Japan. Scanning Electron Microscopy (SEM) Sigma300 (Zeiss) (Germany) and Energy Dispersive X-ray spectroscopy (EDX) system (Oxford) Britain. Inductively Coupled Plasma Spectrometer (ICP) ICPE-9000 (Shimadzu) Japan.

3.3. Green Synthesis of Silver Nanoparticles

BSP (0.5 g) dissolved in deionized water (100 mL) with ultrasonic for 30 min as natural reducing agent and removed the undissolved substance using centrifuge. Silver nitrate (2.5 g) dissolved in deionized water (10 mL) provided silver ions. Finally, tow solutions were mixed in deionized water, and the reaction occurred at 30 °C for 3 h. The color changed from buff to dark brown. Sediment was washed with deionized water 3 times, ethanol 2 times, and dried to brown nano silver.

3.4. Preparation of Silver Nanogel

Carbomer (0.3 mg) was added in batches to glycerol (5 mL) and stirred thoroughly, then a mixture of propylene glycol (3 mL), deionized water (15.4 mL) and 6 mg/mL Ag NPs solution of BSP (0.417 mL) were added. After the mixed gel was fully swollen for 8 h, the gel was defoaming by ultrasound, and the pH of gel was adjusted by triethanolamine to 6.5–7.5.

3.5. Characterization of Nanoparticle

Silver nanoparticles have surface plasmon resonance (SPR) characteristics with characteristic absorption peaks at 300–500 nm. The synthesized silver nanoparticles were dispersed by deionized water, and scanned from a 300 to 600 nm wavelength period using UV. In order to further prove nanoparticle synthesis, Ag NPs dispersed by deionized water were characterized by DLS and TEM. The elemental composition of the nanoparticles was verified by EDX. Additionally, the element distribution of the nanoparticles including Carbon, Oxygen, and Ag were displayed by SEM.

3.6. Antibacterial Activity Analysis

The evaluation of antibacterial activity was performed using the pour plate method (*Staphylococcus aureus*, *Escherichia coli* and *Candida albicans*). In this method, the Ag NPs solution was mixed with a bacterial culture suspension for 5 min. The diluted mixture liquid and an agar culture medium were incubated at 37 °C for 24 h. The petri dishes with a colony number between 30 and 300 were selected to obtain the antibacterial rate.

$$\text{Bacteriostasis rate} \times 100\% = (\text{number of colonies in blank group} - \text{number of colonies in the sample group}) / (\text{number of colonies in blank group}) \times 100\%$$

3.7. Cell Migration In Vitro

NIH/3T3 cells were seeded at a density of 3×10^6 cells/well in 6-well plates with 3 mL of the complete medium (DMEM containing 10% serum) and incubated for 24 h. When the cells were 80% confluent, a scratch straight line was performed on the cell monolayer using pipet tip. The cells in each of the dishes were rinsed twice with PBS. DMEM (3 mL)

containing 2% serum was then supplemented. For the control group, there was only the medium. The test group was 110 μg/mL final concentration BSP. The cells in all of these dishes were incubated at 37 °C and photographed at 0, 24, and 48 h.

4. Conclusions

In this study, a green, environmentally friendly, inexpensive, rapid, and single-step method for the synthesis of nano silver using *Bletilla striata* polysaccharide (BSP) is developed. The morphology and composition were characterized by UV, DLS, ICP and TEM. The antibacterial experiment shows its great antibacterial activity against bacteria including Gram-positive bacteria (*Staphylococcus aureus*), Gram-negative bacteria (*Escherichia coli*) and *Candida albicans*. Our study is meaningful in enriching the green synthesis method of Ag NPs by using BSP, which is synergetic to promote wound recovery.

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Conflicts of Interest: The authors declare no conflict of interest.

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