

Antibacterial and Wound Healing Properties of AgNPs Combined with Other Natural Materials

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Abstract. With the development of new technology industry, nanoparticle is more and more getting valued as one of the most promising technologies in the technological revolution in the 21st century. In recent years, nanotechnology applied in biomedicine is in the ascendant and among the most metal nanoparticles, the silver nanoparticle (AgNP) is a kind of the most common nanomaterials used in wound healing according to its antibacterial properties. For the consideration of biocompatibility and dispersion of the AgNPs, nanocomposites that AgNPs combined with other natural materials like lignin, chitosan, nanofiber and cellulose have been researched a lot to apply to synthesize the wound healing materials. However, there is no sufficient evidence to prove the mechanism of the AgNPs' toxicology until now. Nowadays, AgNPs have been widely used in products, as a result, how to correctly explain the toxicity of AgNPs and the safety to human body and the environment is urgent. In this article, the use of AgNPs combined with other natural materials is reviewed. Furthermore, the possible toxicology which is not mentioned before and the possible solutions are proposed.

Keywords: Silver Nanoparticles, Antibacterial, Wound Healing.

1. Introduction

Nanotechnology is a promising field, with different nanomaterials being manufactured and used in a variety of applications such as medical and pharmaceutical industry, cosmetics, therapy and water treatment. Nanomaterials and nanocomposites have been extensively researched in the past. Due to their high surface area, it shows better physical and chemical properties than original materials with a little amount of use.

Silver nanoparticle (AgNP) is a metal silver element with a diameter of less than 100 nanometers, generally 20 to 50 nanometers. Among most metal elements, silver has been considered as one of most effective used antibacterial substances since thousand years ago. It is widely accepted that the AgNPs can inhibit the growth of bacteria through reacting with the proteins to inactivate enzymes and damage the RNA/DNA by destroying the respiratory chains. Although the antibacterial mechanism of AgNPs has not been proved with sufficient experimental proof, it shows great efficiency and broad application prospects for raw material in wound healing, due to its advantages of long-lasting and broad-spectrum antibacterial properties. You et al. (2017) discovered that a specific amount of AgNPs can help fibroblasts migrate from the edge of the wound. It can promote α -smooth muscle actin to make the enhanced fibroblast to differentiate into myofibroblasts so that it shrinks the wound and promotes faster remodeling of new born tissue [1]. In the last few decades, people's interests in silver element as an antimicrobial agent has risen continuously. Compared to other dressings, silver products show better efficacy of wound healing and they have been already patented and widely commercialized in the wound dressing industry [2].

Wound dressings should have enough gas permeability and a high fluid adsorption capacity to eliminate excessive bacterial-nutrient-containing exudates. It needs to have the ability not only to prevent dangerous bacteria from infecting the wound but also inhibit bacteria's growth and injury to neonatal tissue [3]. It is efficient even with a small number of materials because of the high surface area of the nanoparticles. Furthermore, when compared to traditional antibiotic use, AgNPs will not generate undesirable side effects such as antibiotic resistance or high production costs. With widely use of antibiotics, the variety of pathogens gradually have drug resistance. Besides, the speed of generating drug resistance is faster than production of new antibiotics, which can cause to generate

super bacteria so that it can't be neglected easily. However, it has been proved that very small amount of AgNPs can produce strong antibacterial effect without any drug resistance so that it had been seen as an ideal material for therapeutic purposes and package of antibacterial coatings.

However, considering the metal's toxicology, the AgNPs can be harmful to human body if it is not used in the correct treatment and dose. Therefore, it is a promising research area to develop AgNPs' antibacterial effect with combining with other substances to reduce its toxicology and improve the wound healing effect.

AgNPs are extremely tiny so that they are easy to agglomerate and antibacterial properties will be affected. Bantz, C. et al. (2014) proved that the agglomeration condition of the nanoparticles and their effective size are determined by the surface properties, especially under physiological conditions [4]. It needs a kind of material which can make the AgNPs evenly distributed on the structure. Moreover, for the selected materials, it also requests some specific properties such as high biocompatibility and microporous structure to make the environment not produce any cytotoxic effects to the new born tissue and exchange the substances with the outside world such as oxygen, water and discharged exudates.

A. Gala Morena et al. (2020) found that lignin-capped AgNPs could be added in situ to create multifunctional materials which are appropriate for chronic wound treatment, due to the foams' physical and antibacterial properties. Moreover, their biocompatibility and long-time release of AgNPs make them promising candidates for chronic wound dressings [5]. Bui V.K.H. et al. (2017) researched the chitosan's antibacterial effect when combined with ZnO, TiO₂, Ag nanoparticles to improve the antimicrobial efficiency and application in wound healing. Finally, it was proved that among three nanomaterials, AgNPs seemed to have highest antimicrobial ability in most conditions, although not always [3]. Ye et al. (2016) constructed a cellulose/nanosilver composite aerogel material by freeze-drying to enhance the antibacterial activity and promote the wound healing ability [6]. Cai He et al. (2021) researched and concluded that AgNPs were incorporated into electrospun nanofibers to create a wound dressing whose structure was like sandwich with antimicrobial properties and cytocompatibility, which might be used for wound healing [7]. Although lots of reviews have published AgNPs more than above and some of them are applied in practical production nowadays, the clear toxicology of them is still unclarified. Nanotoxicology has become an emerging subdiscipline in the field of toxicology. To assess the potential toxicity of nanomaterials in products, it needs to understand the potential exposure, material characteristics and potential hazards of nanomaterials at every stage of the products' life cycle. In other words, nanotoxicology is one of the important steps to achieve sustainable development. In this review article, it demonstrates overview and applications of AgNPs combined with four typical kinds of natural materials-lignin, chitosan, nanocellulose and fiber and proposes some perspectives about the nanotoxicology.

2. AgNPs combined with different natural polymer material

2.1. Lignin-capped AgNPs

As one of the components that make up plant cell walls and have the function of connecting cells, lignin is an aromatic polycyclic polymer organic compound containing many negatively charged groups which has a strong affinity for metal ions. Because lignin can be degraded in the nature, lignin nanoparticles could be used as biodegradable substances to eliminate harmful actives with a lower environmental impact than traditional antimicrobial formulations [8]. Due to containing abundant functional groups in Figure 1 [9], including the phenolic, alcoholic hydroxyl, methoxyl, carboxyl groups, etc., lignin has received great attention as a reducing agent when synthesizing AgNPs [10]. Lignin enriched with phenolic groups was employed to decrease silver ions in an enzymatically mediated process, resulting in silver/phenolated-lignin NPs (AgPL NPs) [5]. Moreover, it was proved that the inclusion of lignin on cellulose fibers leads to UV absorption in both the UV-B and UV-C areas, which indicated significantly improved thermal stability. It is widely known that metal

nanoparticles tend to agglomerate more as temperature increases, however, even under high temperature conditions, AgPL NPs remained isolated from one another, without agglomeration [11].

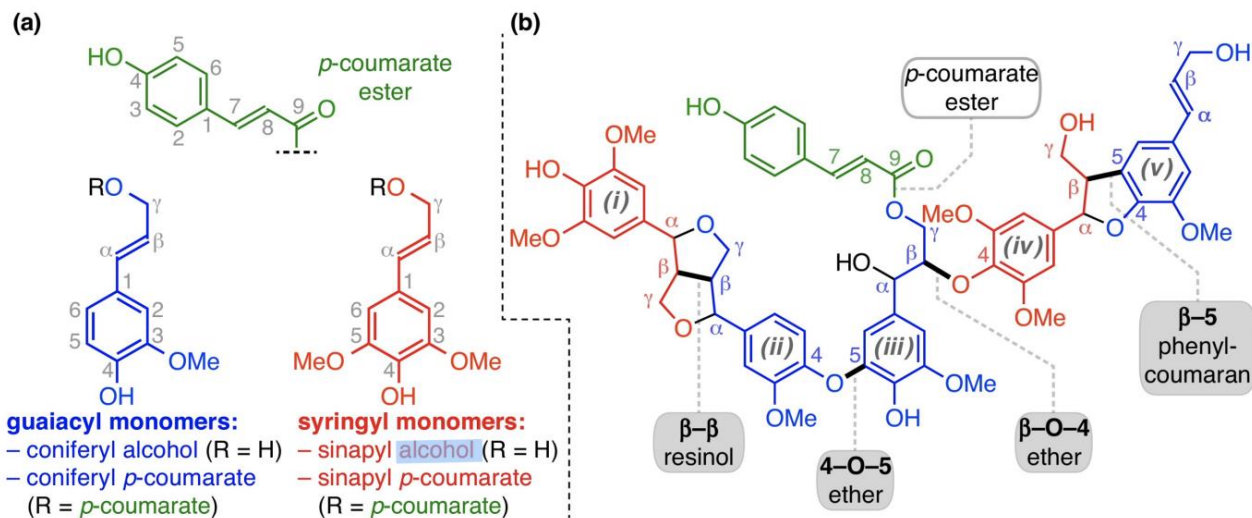


Figure 1. Structure of lignin. (a) The biosynthetic precursors. (b) guaiacyl (blue), syringyl (red) and p-coumarates (green)

Shen et al. (2014) used lignin with reducing groups, stereochemical structure as reducing agent and stabilizer to prepare spherical lignin-based AgNPs with a diameter of 24 nm. The process of mass reduction of Ag^+ is shown in Figure 2 [12]. In the preparation of AgPL NPs, the phenolated lignin was dissolved in the solution with a pH of about 8. It was mixed with $AgNO_3$ and then sonicated for 2 hours at $60^\circ C$. After the centrifugation of the reaction, NPs were purified and further resolved at a low-intensity ultrasound. Then polyurethane foams (PUFs) added with AgPL NPs was prepared. To detect the antibacterial effect of AgNPs, dilution method was used for determining the antimicrobial activity. When foams containing different concentration of AgPL NPs was tested against two clinically relevant bacteria-the gram-positive *S.aureus* and the gram-negative *P.aeruginosa* in Figure 3 [5], results showed that the PUFs added with AgPL NPs are effective through both contacting and releasing mechanisms.

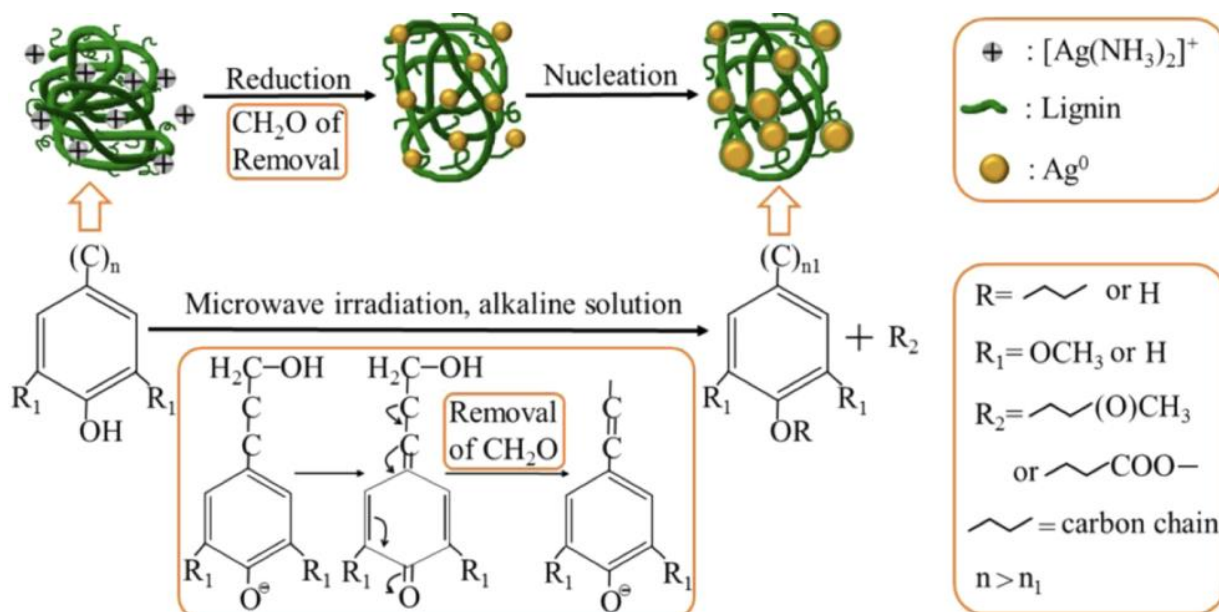


Figure 2. Reduction process when Ag^+ react with lignin

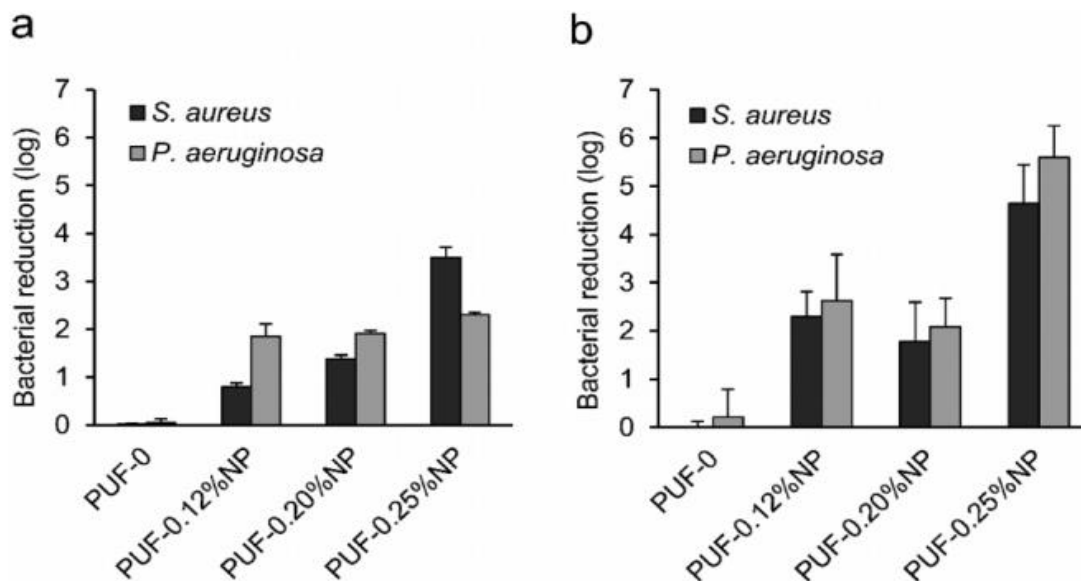


Figure 3. PUFs have antibacterial action against two relevant bacteria. In the release-killing test(a) and in the contact-killing test(b)

Besides, the PUF with greater number of AgNPs have greater antibacterial impact in the range of AgNPs less than 1%. The antibacterial properties of the foams are due to the release of Ag^+ and AgNPs when bacteria come into contacting with them. The open-cell structure of these flexible and resilient foams was observed, with the cell diameter decreasing as the AgPL NPs content increased in Figure 4 [5]. Furthermore, the PUFs' high swelling ratios suggested that they could be able to absorb excessive fluids in severely exudate of the wound. The swelling capacity, depending on the concentration of AgPL NPs, was linked to the ability to absorb the exudate from the wound. Then exudate remove from the wound bed and keep the harmful oxidative species and enzymes within the foam. As a result, it can maintain a moist environment for absorbing the exudate and avoiding drying out. Then researchers were able to create new nano composite foams by combining AgPL NPs into the PUFs, which are ideal for chronic wound treatment.

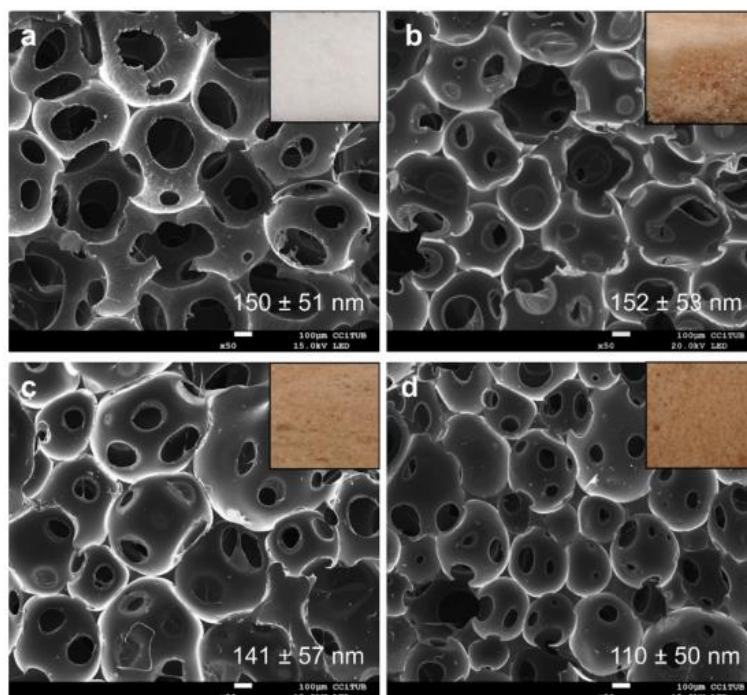


Figure 4. SEM images. PUF with no NPs (a), PUF with 0.12% NPs (b), PUF with 0.20% NPs (c), and PUF with 0.25% NPs (d) at $\times 50$ magnification

Lignin is a rich natural material and is applied in many products in industry such as absorbing toxic metals according to its nanocomposites mechanism when dealing with the water pollution [13]. However, it hasn't been used widely and effectively in biochemical field because if not treated properly, its diversity and the complexity of the chemical structure will inactivate and even harm to the human body. To solve this problem, appropriate biochemical methods and compounding technology should be selected to degrade properly and modify the lignin to increase specific reactive groups which we need.

2.2. AgNPs combined with chitosan

Chitosan is a natural polysaccharide biopolymer. It has the similar chemical structure to the fiber but is more potential as a functional material because the amino group in the molecular structure of chitosan is more activated, which makes the polysaccharide have excellent biological functions and leads to more chemical reactions for modification. The polycationic properties of chitosan are conducive to interacting with microorganisms' cell walls which are negatively charged [14], and further it can cause the disruption of cell membrane functions.

There are some discussions about the toxicity to human health. It has been proved that toxicology depends on the physical properties of nanomaterials used such as the size, shape and the concentration of AgNPs. Chitosan can keep the shape of AgNPs stable [15]. According to the review research done by Bui, V. K. H. et al. (2017), due to the chitosan's biodegradable, non-toxic and antibacterial properties, it has been considered as an ideal material to replace traditional materials in wound healing application. Besides, among lots of metals and their oxides, ZnO NPs, TiO₂ NPS and AgNPs are the most attractive candidates when combined with chitosan. Regarding the antibacterial behavior among these three kinds of nanomaterials, AgNPs seemed to have best antimicrobial effect in most of conditions [3]. The presence of hexagonal AgNPs combined with chitosan revealed that they might stimulate cell growth, prevent from infection, and result in the transfer of small molecules [16]. Ong. SY et al. (2008) researched and produced a composite based on chitosan with polyphosphate and AgNPs. At last, it showed great stanch bleeding and antimicrobial properties. Moreover, the chitosan with AgNPs was also more effective compared to standard gauze treatment [17]. More researches are needed to determine the sorts of wounds that can be successfully treated with silver, reducing the need for systemic antibiotics.

2.3. Cellulose/Ag nanocomposites

Nanocellulose is an ideal material for processing foam and aerogel frameworks because it is a semi-rigid polymer material with the properties of low density, physical stability and thermal stability. Besides, plenty of hydroxyl groups on the surface makes this cellulose rigid and flexible to create a high-performance foam. Nanocellulose materials can not only provide abundant micropores as microreaction reactors to synthesize AgNPs and inhibit their agglomeration, but also provide abundant oxygen-containing groups for grafting active groups. Therefore, nanocellulose can be used to immobilize AgNPs to increase strong antibacterial properties. Besides, cellulose is low cost, biodegradable in ecosystem and biocompatible without skin irritation [18].

Ye et al. [6] constructed antibacterial sponges with the AgNPs with freeze-drying cellulose composite hydrogels. The interconnected pores of the aerogel can be used as a microreactor to synthesize AgNPs and prevent from agglomeration, so that the AgNPs can be released better. By using diffusion method [6, 19], they put the sponges into culture medium and the diameters of the inhibition zones of sponge samples against two kinds of pathogenic bacteria-*S. aureus* and *E. coli* were measured. The results in the Figure 5 [6] confirmed that regenerated cellulose sponges (RCS) without any added AgNPs didn't exhibit antibacterial action against both. However, after 24h incubation, the inhibition zones could be observed around RCS-Ag samples. According to the concentration of the AgNO₃, they researched the antibacterial effect of the modified sponges separately by designing four groups-RCS-Ag1 to RCS-Ag4. With the group number larger, it means the concentration of AgNPs higher. From the results, it could be discovered that when there was no

AgNPs added, it didn't exhibit the inhibition circle. And with the increase of the AgNPs, the diameter of the antibacterial circle was more and more big. Finally, they could draw a conclusion that the concentration of AgNPs was linked to the antibacterial activity of the modified sponges.

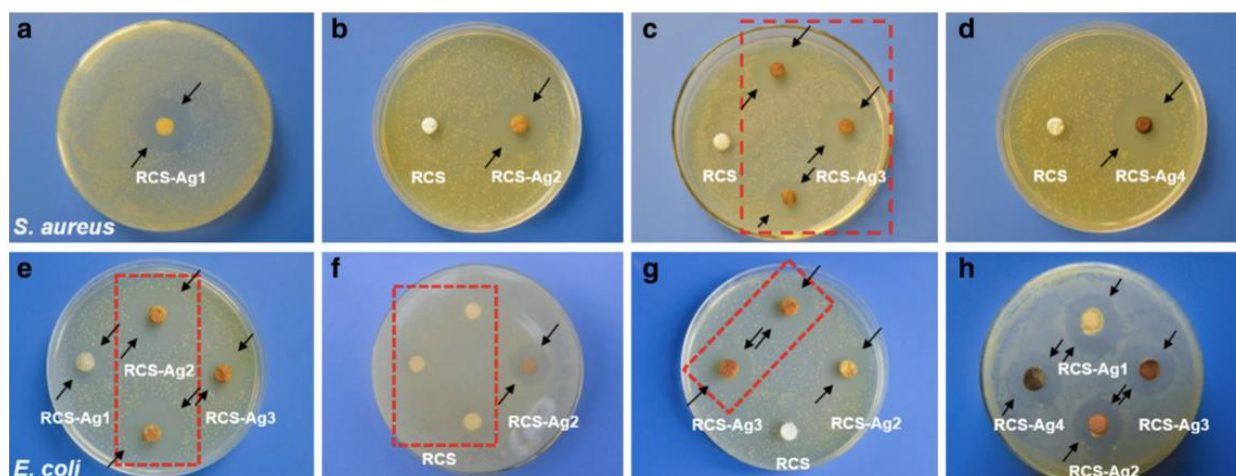


Figure 5. Antibacterial circle of the five different experiment groups against

S. aureus (a–d) and *E. coli* (e–h) after 24h's incubation and comparison between them

To examine the infected wound healing efficiency of modified sponges in vivo, they used the New Zealand rabbit model. The findings showed that the RCS with a specific amount of AgNPs can control infection and prevent the wound from being infected with a purulent infection. Furthermore, it supported recuperation more effectively than gauze. As a result of the findings, it was determined that the modified sponges have the ability to accelerate the healing of infected wounds [6].

In recent years, nanocellulose has been increasingly used in bio-based materials, and the combination with AgNPs can make composites have long-lasting antibacterial properties. It is necessary to develop low-cost, environmental, safe, efficient and broad-spectrum antibacterial materials. To promote and apply cellulose antibacterial materials, it is also necessary to improve the depth and breadth of research. Therefore, before clinical application, it is necessary to do additional experiments, such as testing the effect of medical antibacterial dressings on humans. According to the chemical reactivity of nanocellulose surface groups, it is necessary to select appropriate modification methods to stably combine AgNPs and nanocellulose. Then the sustained release of silver can be effectively controlled during use, which can further improve the use of materials and security.

2.4. Nanofibers and AgNPs

Cai He et al. (2021) discovered that electrospun nanofiber nonwoven mats have a lot of potential for wound healing because of their structural reproduction of native extracellular matrix [7]. The wound dressings outside layer were hydrophobic, which inhibited external microbe adhesion and invasion. Invading microorganisms could be killed by a specially engineered intermediate region with a high concentration of AgNPs. To stimulate cell growth, a hydrophilic surface of the polycaprolactone/gel was employed in the inner layer adhered to the wound bed. This layer also worked as a screen to prevent cytotoxicity from the abundant discharge of AgNPs in the wound's immediate vicinity [7]. The design and manufacture of the sandwich-structured wound dressing can keep a balance of the antimicrobial activity and biocompatibility between the fiber and AgNPs and mix them appropriately. Kashid et al. (2017) reported the approach of biogenic and situreduction of Ag^+ on the cellulosic fiber is more biocompatible, authentic, and reliable than traditional methods, which is necessary when producing a new material used in wound healing [20]. The dispersed spherical agglomerations of AgNPs with individual particle sizes approximately 50 nm were discovered. Besides, the cotton–AgNPs fibers performed well in antimicrobial tests against pathogenic bacteria and fungi. Tanja et al. (2017) introduced a new green in-situ procedure of AgNPs

onto and into cellulose fibers. By analysis of scanning electron microscopy (SEM) and energy dispersive x-ray spectroscopy, they imagined the coatings' morphology and determination of spatial presence of AgNPs. In addition, even after 20 washing cycles the modified fibers have outstanding persistence of particles' coating against washing and excellent antimicrobial properties. Moreover, the moisturizing effect of Ag-treated fibers was also improved, while mechanical properties were not noticeably harmed [21].

Sovan et al. (2019) found the AgNPs based nature rubber latex (NPL) revealed superior antibacterial property against microorganisms. Therefore, it can be concluded that AgNPs incorporated NPL coating over the cotton fiber and this kind of modified cotton fiber can have a great potential prospect in biomedical industry [22]. Wang Qiuxiang et al. (2022) used researched coaxial electrostatic spinning technique to investigate a nanofiber membrane loaded with curcumin and AgNPs. From the antibacterial tests, it could be concluded the nanofiber membrane modified with curcumin and AgNPs had outstanding antibacterial effects on both *S. aureus* and *E. coli* with antibacterial rates over 90% [23].

3. Conclusions

In short, the AgNPs have been used in many products and techniques in various different forms nowadays, according to their antibacterial effects in pharmaceutical industry, implantation of medical devices and especially wide used in wound healing. The development of the green antibacterial agent and green synthesis process are more and more important to achieve both development of industry and reduce lower pollution to coordinate with the nature. Although there are some discussions about the toxicity to human health, it depends on the several factors of nanomaterials used such as the size, surface, morphology and agglomeration condition. Besides, AgNPs combined with other natural substances can decrease the toxicity and improve more functions. However, most toxic substances can enter the body through the skin. The epidermis of the skin has no blood vessels so nanoparticles can escape from the clearance of phagocytes and remain on the surface of the skin, while wounds are more fragile and sensitive, and have lower immunity. Compared with the traditional healing method, nanoparticles are more difficult to control and assess its toxicology because they contact the skin as an aggregation of molecules and even atoms, which also means it can't be discovered easily and danger is potential. Therefore, the concentration and size of AgNPs should be paid more attention to and well controlled when preparing wound healing materials to prevent accumulation in the body and damage to health. Besides, from a macro perspective, before applying to practical use, plenty of biological experiments and clinic trials are the most essential. So, research further for using the AgNPs and their modifications as a new ideal type of materials is an important direction. Although the results of studies of AgNPs toxicity are largely inconsistent. Most of the drugs made of AgNPs rely on their antimicrobial properties. Though its toxic effects cannot be ignored, they should also not be overstated. It is necessary to clarify the risk which the AgNPs bring to human health and environment protection so that the research on the basic theory should be increased to provide more theoretical support for the multifunctionalization and industrialization of antibacterial materials. AgNPs combined with other natural materials can play a complementary role with each other to further increase the load broad-spectrum and high-efficiency persistence of antibacterial properties if designed in a proper way. In the future, we can use various microscope to examine the change of morphology and DNA damage to bacterial cells to figure out potential antibacterial mechanism from a micro perspective. The development of antibacterial materials used in wound healing and even more products would be aided by the increase of antibacterial effect against drug-resistant bacteria.

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