Antibacterial and antibiofilm effects of gold and silver nanoparticles against the uropathogenic *Escherichia coli* by scanning electron microscopy (SEM) analysis

Rini Purbowati,[1] Vania Mitha Pratiwi,[2] Masfufatun Masfufatun,[3] Putu Oky Ari Tania,[1] Ali Khumaeni[4]

[1]Biomedical Department and Biomolecular Research, Faculty of Medicine, Universitas Wijaya Kusuma Surabaya, Surabaya; [2]Department of Materials and Metallurgical Engineering Faculty of Industrial Technology and Systems Engineering, Institut Teknologi Sepuluh Nopember, Surabaya; [3]Department of Biochemistry, Faculty of Medicine, Universitas Wijaya Kusuma Surabaya, Surabaya; [4]Department of Physics, Faculty of Science and Mathematics, Universitas Diponegoro, Semarang, Indonesia

Rini Purbowati, Biomedical Department and Biomolecular Research, Faculty of Medicine, Universitas Wijaya Kusuma Surabaya, Surabaya, Indonesia. E-mail: rini.purbowati@uwks.ac.id

Key word: UPEC; biofilm; antibiotic resistance; nanoparticles; SEM analysis.

Contributions: RP, Conceptualization, Data Curation, Formal Analysis, Methodology, Validation, Visualization, Writing – Original Draft, Review & Editing; Conceptualization, Investigation, Methodology, Validation, Writing – Original Draft, and Review & Editing; POAT, Conceptualization, Methodology, Formal Analysis, Validation, and Writing – Original Draft, Review & Editing; Methodology, Visualization, Writing – Review & Editing; AK, Resources, Investigation, and Writing – Review & Editing; MM Formal Analysis, Validation, Writing – Review & Editing; Resources, Supervision, and Writing – Review & Editing; VMP, Resources, Investigation, and Writing – Review & Editing.

Conflict of interest: the authors declare no conflict of interest.

Ethics approval and consent to participate: the conducted research is not related to either human or animals use.

Funding: this research was supported by a research grant from Direktorat Jenderal Penguatan Riset dan Pengembangan, Kementerian Riset, Teknologi dan Pendidikan Tinggi with contract number 183/E5/PG.02.00.PL/2023.

Availability of data and materials: all data generated or analyzed during this study are included in this published article.

Acknowledgement: we would like to thankful to Institute of Tropical Disease, Airlangga University, Surabaya for their valuable insights and contributions to this study.

Received: 11 September 2023.
Accepted: 18 October 2023.
Early access: 26 October 2023.

This work is licensed under a Creative Commons Attribution 4.0 License (by-nc 4.0).

©*Copyright: the Author(s), 2023*
Licensee PAGEPress, Italy
Healthcare in Low-resource Settings 2023; 11:11748
doi:10.4081/hls.2023.11748

Publisher's note: all claims expressed in this article are solely those of the authors and do not necessarily represent those of their affiliated organizations, or those of the publisher, the editors and the reviewers. Any product that may be evaluated in this article or claim that may be made by its manufacturer is not guaranteed or endorsed by the publisher.

Abstract

Uropathogenic Escherichia coli (UPEC) is a nosocomial pathogen associated with urinary tract infections and biofilm formation, which contributes to antibiotic resistance. Discovering potent antibacterial agents is crucial. This study aimed to assess the antibacterial and antibiofilm effects of gold and silver nanoparticles on UPEC using Scanning Electron Microscopy (SEM). UPEC biofilms were cultivated on nitrocellulose membranes for 48 hours at 37°C, then treated with gold nanoparticles (50 ppm and 100 ppm) and silver nanoparticles (50 ppm and 100 ppm) for another 48 hours. Antibacterial and antibiofilm activities were evaluated through cell density and SEM analysis. SEM revealed lower cell density, reduced biofilm formation, and altered cell morphology with rough, wrinkled surfaces after nanoparticle treatment. In conclusion, gold and silver nanoparticles exhibit antibacterial and antibiofilm properties, as observed in SEM analysis. SEM is a valuable tool for studying the antimicrobial effects of nano gold and silver on bacterial cell morphology and biofilm populations.

Introduction

Urinary tract infections (UTIs) affect approximately 150 million people annually worldwide, leading to significant healthcare expenditures. UTIs are the most prevalent bacterial infections and are considered a critical health issue, following respiratory and digestive tract infections.[1,2] These infections are more common in women due to factors such as fecal flora contamination, the shorter female urethra, and pregnancy. UTIs affect individuals across various age groups, including neonates, young women, infants, children, and older men.[3] *Escherichia coli* (E. coli) is the predominant pathogen, causing over 80-90% of community-acquired UTIs and 30-50% of hospital-acquired UTIs.[4,5] *Uropathogenic Escherichia coli* (UPEC) is a nosocomial pathogen associated with UTIs. UPEC utilizes various cellular appendages, including fimbriae and pili, to colonize and adhere to the bladder, forming biofilm-like bacterial communities. These biofilms play a crucial role in sustaining UPEC's survival and evading the host's immune response.[6,7] The ability to adhere to epithelial cells, resist urine flow, and form biofilms are key factors that make UPEC the primary cause of UTIs in humans.[8]

Biofilms are estimated to be responsible for about 65% of nosocomial infections and 80% of all microbial infections.[9] These structured microbial communities, enveloped in an extracellular matrix (ECM), adhere to various surfaces. Biofilm-associated cells exhibit distinct phenotypic characteristics compared to

Non-commercial use only

planktonic or motile cells. Notably, biofilms exhibit significantly higher resistance to antimicrobial agents, with microbial biofilms formed through the attachment of bacteria using a secreted polymer matrix. The primary constituents of this matrix include extracellular DNA, proteins, and polysaccharides.[10,11] Biofilm-embedded cells generally display greater tolerance to antibiotics and the host's immune system, with biofilm resistance to antibiotics being 100-1000 times higher than planktonic cells.[12]

The increasing prevalence of antibiotic-resistant bacteria is a global concern, as highlighted by the World Health Organization. Moreover, the limited solubility, stability, and adverse side effects associated with current antibacterial therapies have prompted researchers to seek innovative strategies to combat these resilient microbes.[13,14] This has led to a growing demand for new antibiotic delivery systems. Nanotechnology, with its advantageous physicochemical properties, drug-targeting efficiency, enhanced absorption, and biodistribution, has gained significant attention.[15] Antibacterial research is a thriving field within nanomedicine, aimed at meeting drug delivery requirements, reducing antibiotic concentrations, and curbing drug resistance among pathogenic bacteria.[16] Numerous studies have demonstrated the antibacterial and antibiofilm activities of gold and silver nanoparticles against antibiotic-resistant bacteria. For instance, gold nanoparticles (AuNPs) have shown superior antibacterial potential compared to crude ethanol extracts of *Digera muricata* against various drug-resistant bacteria, including *Vibrio cholera, Staphylococcus pyrogen, Klebsiella, Citrobacter,* and *Enterobacter.*[17,18] Similarly, silver nanoparticles (AgNPs) derived from *Ferula ovina Boiss* (FOB) extracts exhibited effective antibacterial activity against both Gram-positive (*Staphylococcus aureus* and *Bacillus cereus*) and Gram-negative (*Salmonella typhimurium* and *Escherichia coli*) species using the disk diffusion method.[19] Ginger AgNPs demonstrated potent antibacterial and anti-adherent activity against biofilm-associated enterococcal isolates.[20] Furthermore, AgNPs exhibited significant dose-dependent antibiofilm activity, reducing biofilm formation at concentrations of 20 and 10 g/ml. When exposed to 20 g/ml of AgNPs, *S. pseudintermedius* displayed an uneven biofilm surface, indicating biofilm aggregation.[21]

Regrettably, previous study did not investigate cellular morphology changes to explain alterations in cell surface structures and biofilm visualization. Scanning electron microscopy (SEM) is a valuable tool for visualizing biofilms and providing accurate descriptions of biofilm morphology. Comparative analyses, such as evaluating the anti-biofilm effects of treatments, are highly useful because SEM imaging results strongly correlate with findings from other analytical methods. SEM micrographs have been employed to observe changes in the bacterial plasma membrane of drug-resistant *S. aureus* and *P. aeruginosa* cells following treatment with Macropin, a novel antimicrobial agent.[22] The aimed of this study was to evaluate the antibacterial and antibiofilm effects of gold and silver nanoparticles against UPEC through SEM analysis.

Materials and Methods

Bacterial isolates and nanoparticles (gold and silver)

The UPEC strain used in this study was obtained from a previous isolation study.[23,24] The research indicates that this particular UPEC strain is capable of forming biofilms, as determined by the microtiter plate method. The bacterial strains were cultured on Eosin Methylene Blue (EMB) agar at 37°C for two days. This specific strain was originally isolated from patients suffering from UTIs and subsequently processed at the Gastroenteritis and Salmonellosis laboratory, Institute of Tropical Disease, Airlangga University, Surabaya, Indonesia. The gold and silver nanoparticles used in this study were provided as finished products by the Nanotechnology Laboratory at Diponegoro University.[25]

Preparation of UPEC inoculum

The UPEC strain was initially grown on nutrient agar (NA) medium for 24 hours at 37°C. A subculture of the UPEC was then cultivated on Luria Bertani (LB) medium for an additional 24 hours at 37°C. Following incubation, the culture was centrifuged at 5000 rpm for 5 minutes. The supernatant was subsequently resuspended in 0.9% NaCl and adjusted to an optical density (OD_{490}) of 0.5, equivalent to approximately 10^8 CFU/mL. This prepared inoculum was used in each treatment. The gold and silver nanoparticles were sourced from the Nanotechnology Laboratory at Diponegoro University.

Biofilm analysis of nitrocellulose membrane using SEM

As previously research finding described,[26] the UPEC inoculum was applied to a nitrocellulose membrane and allowed to grow for 48 hours. The cultures were incubated at 37°C. Following the biofilm formation, it was treated with gold nanoparticles (50 ppm and 100 ppm) and silver nanoparticles (50 ppm and 100 ppm), respectively, and incubated for an additional 48 hours at 37°C. The processed biofilm was then dried using an oven at a temperature of 36-37°C for 12 hours. Dry membranes were dehydrated by immersion in ethanol with varying concentrations: 50% for 10 minutes, 70% for 10 minutes, and 96% for 20 minutes. The process was completed by coating the samples with gold, making them ready for analysis using a SEM (FEI Inspect S50). Antibacterial activity was assessed by evaluating cell density, while antibiofilm activity was analyzed descriptively by examining the appearance of the slime covering the cell population in SEM images.

Results

Figure 1 displays a representative image of UPEC bacterial cells undergoing growth and biofilm formation on a nitrocellulose membrane (A-B) in the negative control, and with the addition of the antibiotic Chloramphenicol (C-D) as a positive control after 24 hours of incubation. In Figure 1A, the cell density is high, with cell colonies (lighter color) evenly covering the surface of the nitrocellulose membrane (darker color). The cells appear intact and maintain a smooth surface, indicating that the cell membranes are not contracted, and the cell morphology remains undistorted. The biofilm formation is evident as a slimy layer, reducing the visibility of elliptical cell shapes, causing bacterial cells to cluster. In Figure 1B, the cell density is significantly lower, with cell colonies (lighter color) visible over a smaller portion of the nitrocellulose membrane (darker color). The biofilm formed is less pronounced, enabling the elliptical cell shapes to be more discernible, and the bacterial cells show a tendency to remain separate.

Figure 2 depicts a representative image of UPEC bacterial cells exposed to gold nanoparticles at concentrations of 50 ppm (B-C) and 100 ppm (E-F) after 24 hours of incubation. In Figure 2B, the cell density is lower in comparison to the control (Figure 2A). While the growth of cell colonies is still generally evenly distributed across the

Non-commercial use only

OPEN ACCESS

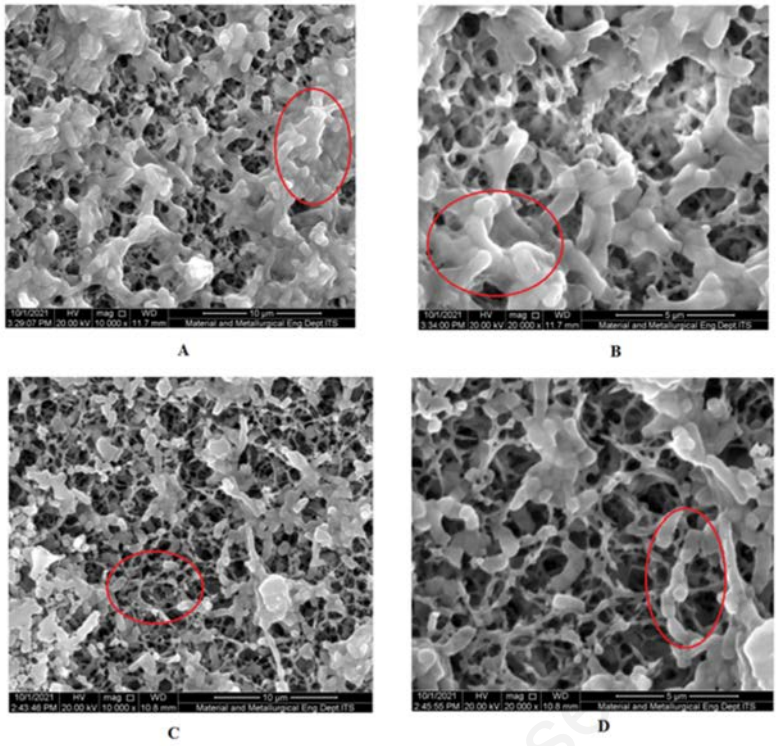

Figure 1. Scanning electron microscopic analysis of biofilm structure. SEM images of biofilm formed on nitrocellulose membrane after 24 h of incubation. (**A**) Negative control in 10.000 x, (**B**) Negative control in 20.000 x, (**C**) Positive control in 10.000 x, (**D**) Positive control in 20.000 x.

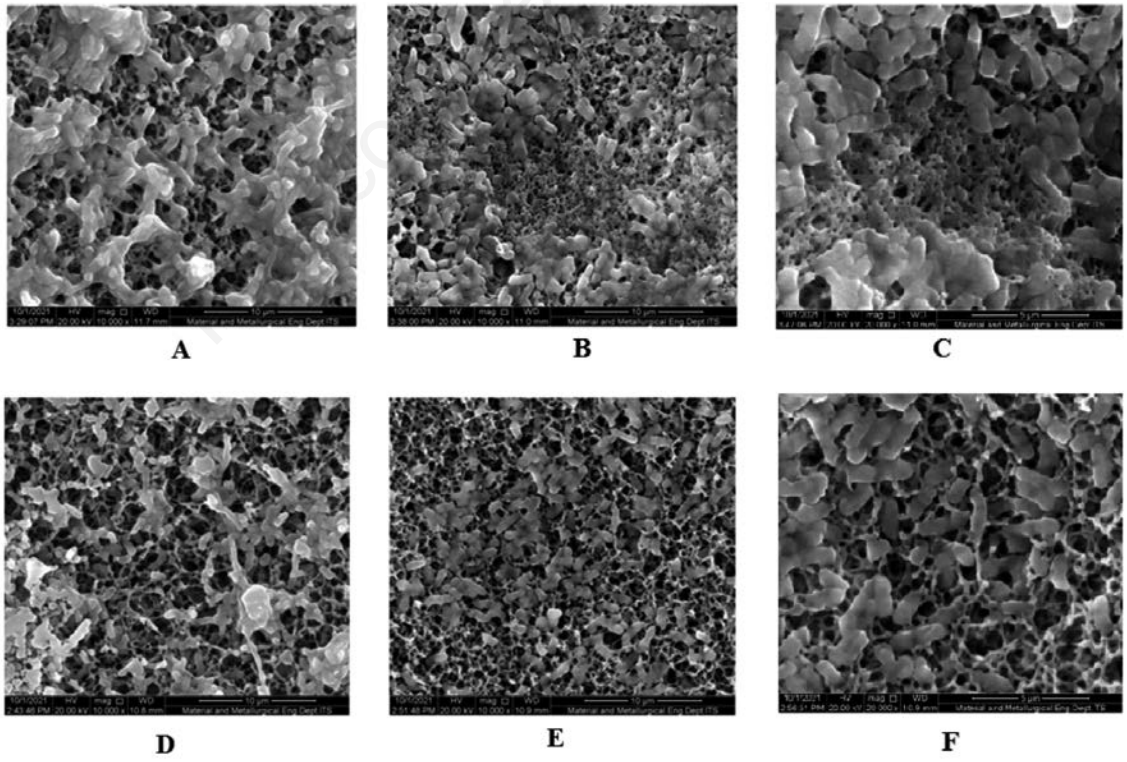

Figure 2. Scanning electron microscopic analysis of biofilm structure. SEM images of biofilm formed on nitrocellulose membrane treated with gold nanoparticle after 24 h of incubation. (**A**) Negative control in 10.000 x; (**B**) treated with 50 ppm in 10.000 x; (**C**) treated with 50 ppm in 20.000 x; (**D**) treated with 100 ppm in 10.000 x; (**E**) treated with 100 ppm in 20.000 x; and (**F**) Positive control in 10.000 x.

membrane surface, the formed biofilm is still visible. However, noticeable alterations in morphology and cell surface are observed. The cells appear intact and maintain a rough and wrinkled surface, suggesting contracted cell membranes and distorted cell morphology (Figure 2C). In Figure 2D-E, the cell density is notably lower, and the biofilm formed is significantly reduced, causing bacterial cells to fragment. The cells remain intact and retain a rough and wrinkled surface, indicating membrane structure damage.

Figure 3, which presents results similar to those in Figure 2, demonstrates the response of UPEC bacteria when exposed to silver nanoparticles at concentrations of 50 ppm (B-C) and 100 ppm (E-F) after 24 hours of incubation. In Figure 3B, the cell density is lower compared to the control (Figure 2A). The growth of cell colonies is still evenly distributed across the membrane surface. The biofilm is still apparent, yet noticeable alterations in morphology and cell surface are observed. Cells maintain their integrity but exhibit a rough and wrinkled surface, indicative of contracted cell membranes and distorted cell morphology (Figure 3C). In Figure 3D-E, the cell density is considerably lower, and the biofilm formed is substantially reduced, causing bacterial cells to fragment. The cells appear intact with a rough and wrinkled surface, highlighting damage to the membrane structure.

Discussion

Nanotechnology has emerged as a significant and increasingly intriguing field of research over the last three decades. Its applications span various sectors, with substantial focus on the medical field, encompassing diagnostics, therapeutic tools, and biomedical research. This amalgamation of nanotechnology with the realm of human health is referred to as nanomedicine.[27] Nanomaterials have demonstrated considerable potential in revitalizing the antibacterial activity of conventional antibiotics through mechanisms that include optimizing pharmacokinetics, enhancing antibiotic internalization, disrupting bacterial metabolism, increasing biofilm penetration, and modifying the biofilm microenvironment.[28] The amalgamation of nanotechnology and antibiotics presents the most promising strategy for combating bacterial resistance to antibiotics.[29] Moreover, emerging antimicrobial nanomaterials are evolving into nanomedicines, wielding a wide-ranging impact on biomedical applications, encompassing targeting, imaging, therapy, and beyond.[30]

Numerous studies have showcased the antibacterial efficacy of gold and silver nanoparticles against both Gram-positive and Gram-negative bacteria. For instance, GCL AgNPs exhibited a significant inhibition zone, with a diameter of 12.2 mm, against *S. enterica*, followed by an 11.8 mm diameter zone against *P. aeruginosa*.[31] Green-synthesized silver nanoparticles exhibited potent activity against foodborne pathogenic bacteria and displayed the potential to combat Gram-negative and Gram-positive bacteria.[32] Furthermore, the antibacterial potency of synthesized BV@AgNPs was examined against seven clinically isolated multidrug-resistant bacteria. The Minimum Inhibitory Concentration (MIC) values of Berberis vulgaris (BV)@AgNPs against various bacteria were established, revealing their high antibacterial activity.[33] The application of AuNPs extends to diverse fields, including therapy, medicine, and pharmaceutical.[35]

Studies have also demonstrated the antibiofilm properties of

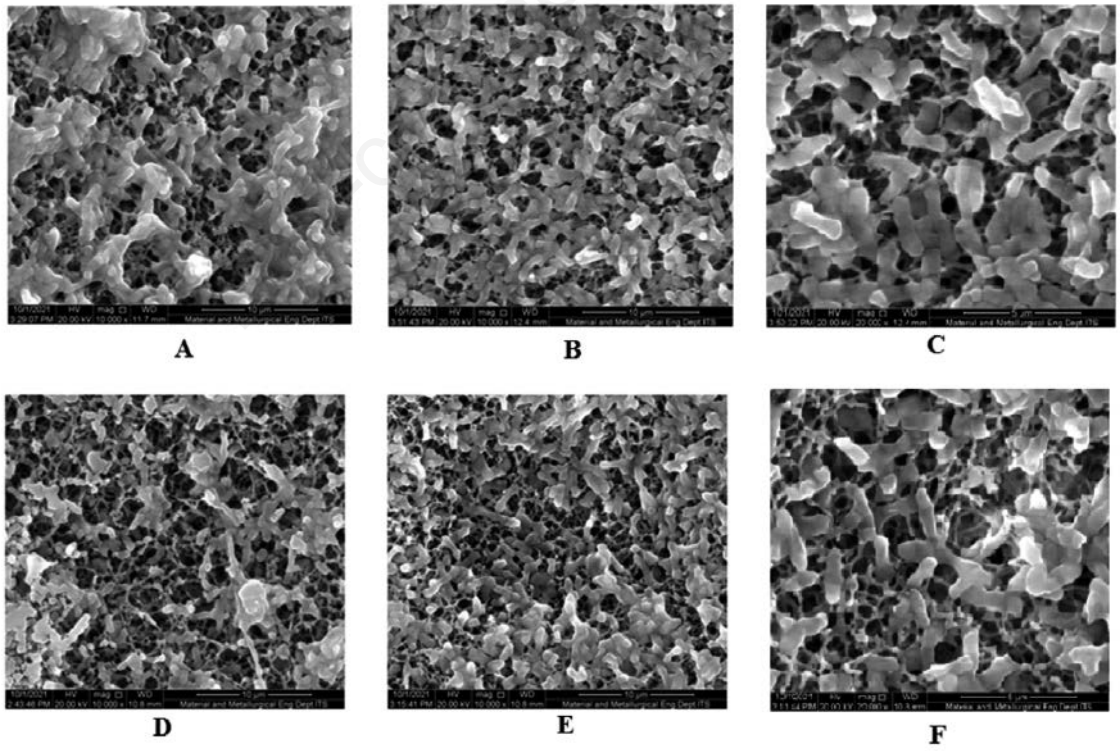

Figure 3. Scanning electron microscopic analysis of biofilm structure. SEM images of biofilm formed on nitrocellulose membrane treated with silver nanoparticle after 24 h of incubation. (**A**) Negative control in 10.000 x; (**B**) treated with 50 ppm in 10.000 x; (**C**) treated with 50 ppm in 20.000 x; (**D**) treated with 100 ppm in 10.000 x ; (**E**) treated with 100 ppm in 20.000 x; and (**F**) Positive control in 10.000 x.

gold and silver nanoparticles against both Gram-positive and Gram-negative bacteria, shedding light on economical methods of AgNP production with specific properties to target the growth modes of pathogenic *C. Albicans.*[36] These findings underscore the safety and effectiveness of AgNPs against MDR *K. Pneumoniae.*[37] The precise mechanisms underlying the antibacterial activity of nanoparticles are not yet fully understood, but it is believed to be attributed to one or a combination of mechanisms, such as the production of reactive oxygen species (ROS), the release of toxic ions, and the direct interaction of deleterious particles with cell membranes.[38] Direct contact may induce stressful stimuli through electrostatic interactions between nanoparticles and bacterial cell surfaces, leading to ROS production and bacterial cell demise.[39] The disruption of the cell membrane, causing intracellular content leakage, is another facet of the antibacterial activity of nanoparticles. It's worth noting that the antibacterial activity of NPs varies based on the cell composition of specific bacteria; Gram-positives are more susceptible to the antimicrobial action of ZnO due to differences in cell wall thickness and other components.[16] The overall mechanical properties of bacteria are influenced by the characteristics of their cell envelope, including its integrity, and various factors like natural lytic elements. Biochemical composition, conformational properties, and biomolecule density in the cell envelope play vital roles in determining bacterial elasticity, with the peptidoglycan layer prominently impacting cell elasticity.[40]

TC-AuNPs demonstrated a dose-dependent reduction in the ability of *P. aeruginosa* to form biofilms, as revealed by SEM analysis. A higher concentration of nanoparticles was associated with a decreased number of biofilm-forming cells, indicating reduced adhesion and colonization on the surface. However, it is important to note the inherent limitations of SEM analysis, such as challenges in detecting extracellular polymeric substances (EPS) and reductions in total cell volume and architecture due to SEM's dehydration process.[41] The biological impact of AgNPs relies on several mechanisms, including binding to the cell wall, which alters permeability. For example, in studies on Gram-negative bacteria like *E. coli* and *P. aeruginosa*, AgNPs neutralized the bacterial surface charge, affecting membrane permeability. Scanning and transmission electron microscopy demonstrated that AgNPs could create holes in the cell wall, leading to AgNP accumulation.[42] For a more comprehensive exploration of the antibacterial mechanism and bacterial morphology changes, SEM was employed to visualize *S. aureus* and *E. coli* cells. The study yielded results consistent with previous findings.[43] Prior to treatment, bacterial cells exhibited smooth, intact membranes and normal morphology. After exposure to AgNPs, cells displayed deformities, disorganization, and surface cavities. The NPs adhered to the cell surface due to electrostatic attraction between the bacterial cell surface and the NPs. Aggregation of NPs was more pronounced and rapid in *E. coli*, potentially due to differences in cell wall composition between Gram-positive and Gram-negative bacteria. The outcome was the disruption of the outer membrane and deformities in cellular structures, leading to penetration into bacterial cells and interference with essential functions.[44]

Biogenic AgNPs demonstrated the potential to inhibit the growth of pathogens, particularly well-structured bacterial biofilms like UPEC. The differences in biofilm structure among bacterial species and the physicochemical properties of AgNPs are significant factors affecting the efficacy of their antibiofilm activity. UPEC formed planktonic, preformed, and mature biofilms, suggesting that bacterial aggregation and physiology play pivotal roles in determining the mechanisms behind AgNPs' antibacterial activity. These mechanisms may involve increased oxidative stress resulting from intracellular Ag+ ion production, changes in membrane potential and respiratory chain function, and interactions with DNA and regulatory proteins.[44] Biofilm formation is a multifaceted microbial process involving distinct developmental stages specific to different bacterial types.[45] These biofilms are held together by extracellular polysaccharides, proteins, and nucleic acids, and biofilm development in E. coli serves a crucial role in disease causation and induction. Biofilm formation is a complex process with a marked structure that aids in the storage of antimicrobial peptides, reducing corrosion. Residual bacterial biofilms pose a significant health risk, characterized by their resilience to treatment and potential for nosocomial transmission. Thus, the exploration of natural molecules to address these substantial challenges, and the ability of antibacterial agents to deter biofilm formation or destruction, remains an area of great importance.[46]

Conclusions

This study demonstrates the antibacterial and antibiofilm activities of gold and silver nanoparticles, as evident from the SEM analysis. SEM proves to be an invaluable tool for in-depth investigations into the antimicrobial properties of nano gold and silver on bacterial cell morphology and biofilm populations. SEM can serve as an essential tool for assessing the efficacy of antibiotic and antibiofilm agents in microbial infections. Future research may explore other analytical methods, including Confocal Laser Scanning Microscopy (CLSM) and Transmission Electron Microscopy (TEM).

References

1. Sari YIP, Rohmah UN, Andini SA, Luwao HP. Summary of the Prevention of Catheter-Associated Urinary Tract Infection in An Intensive Care Unit. Jurnal Ners 2019;14:103–7.
2. Javed S, Mirani ZA, Pirzada ZA. Phylogenetic Group B2 Expressed Significant Biofilm Formation among Drug Resistant Uropathogenic Escherichia coli. Libyan J Med 2021;16:1845444.
3. Issa OM, Bakir WAE, Abbas MA. Laboratory diagnosis of urinary tract infections in patients with resistance genes towards antibiotics. Bionatura 2022;7:46.
4. Sjahriani T, Wasito EB, Tyasningsih W. Isolation and Identification of Escherichia coli O157:H7 Lytic Bacteriophage from Environment Sewage. Int J Food Sci 2021;2021:7383121.
5. Katongole P, Nalubega F, Florence NC, et al. Biofilm formation, antimicrobial susceptibility and virulence genes of Uropathogenic Escherichia coli isolated from clinical isolates in Uganda. BMC Infect Dis 2020;20:1-6.
6. Syaiful I, Widodo ADW, Endraswari PD, et al. The association between biofilm formation abilitand antibiotic resistance phenotype in clinical isolates of gram-negative bacteria: a cross-sectional study. Bali Med J 2023;12:1014-20.
7. Boya BR, Lee JH, Lee J. Antibiofilm and Antimicrobial Activities of Chloroindoles Against Uropathogenic Escherichia coli. Front Microbiol 2022;13:872943.
8. Magtoto R, Poonsuk K, Baum D, et al. Evaluation of the Serologic Cross-Reactivity between Transmissible Gastroenteritis Coronavirus and Porcine Respiratory Coronavirus Using Commercial Blocking Enzyme-Linked

Non-commercial use only

Immunosorbent Assay Kits. mSphere 2019;4:e00017-19.
9. Bharadwaj KK, Rabha B, Choudhury BK, et al. Current strategies in inhibiting biofilm formation for combating urinary tract infections: Special focus on peptides, nano-particles and phytochemicals. Biocatal Agricult Biotechnol 2021;38:102209.
10. Yang YM, Osawa K, Kitagawa K, et al. Differential effects of chromosome and plasmid blaCTX-M-15 genes on antibiotic susceptibilities in extended-spectrum beta-lactamase-producing Escherichia coli isolates from patients with urinary tract infection. Int J Urol 2021;28:623-8.
11. Sonawane JM, Rai AK, Sharma M, et al. Microbial biofilms: Recent advances and progress in environmental bioremediation. Sci Total Environ 2022;824:153843.
12. Olar R, Badea M, Chifiriuc MC. Metal Complexes-A Promising Approach to Target Biofilm Associated Infections. Molecules 2022;27:758.
13. Mhawesh A, Khudair M, Abbas ON. Major Genetic Determinants of Extended-Spectrum β-Lactamase (ESBL), Carbapenemase, Fosfomycin and Colistin Resistance in Escherichia Coli from Intensive Care Units. Bionatura. 2022;7:11.
14. Waskito LA, Yamaoka Y. The Story of Helicobacter pylori: Depicting Human Migrations from the Phylogeography. Adv Exp Med Biol 2019;1149:1-16.
15. Eleraky NE, Allam A, Hassan SB, Omar MM. Nanomedicine Fight against Antibacterial Resistance: An Overview of the Recent Pharmaceutical Innovations. Pharmaceutics 2020;12:142.
16. Zubair N, Akhtar K. Morphology controlled synthesis of ZnO nanoparticles for in-vitro evaluation of antibacterial activity. Trans Nonferrous Metals Soc China 2020;30:1605-14.
17. Sathiyaraj S, Suriyakala G, Dhanesh Gandhi A, et al. Biosynthesis, characterization, and antibacterial activity of gold nanoparticles. J Infect Public Health 2021;14:1842-7.
18. Shah R, Shah SA, Shah S, Faisal S, Ullah AF. Green synthesis and antibacterial activity of gold nanoparticles of digera muricata. Indian J Pharmaceut Sci 2020;82:374-8.
19. Allafchian A, Vahabi MR, Jalali SAH, et al. Design of green silver nanoparticles mediated by Ferula ovina Boiss. Extract with enhanced antibacterial effect. Chem Physics Lett 2022;791:139392.
20. Swidan NS, Hashem YA, Elkhatib WF, Yassien MA. Antibiofilm activity of green synthesized silver nanoparticles against biofilm associated enterococcal urinary pathogens. Sci Rep 2022;12:3869.
21. Seo M, Oh T, Bae S. Antibiofilm activity of silver nanoparticles against biofilm forming Staphylococcus pseudintermedius isolated from dogs with otitis externa. Vet Med Sci 2021;7:1551-7.
22. Rusu D, Stratul SI, Calniceanu H, et al. A qualitative and semiquantitative SEM study of the morphology of the biofilm on root surfaces of human teeth with endodontic-periodontal lesions. Experim Therapeutic Med 2020;20:201.
23. Kitagawa K, Shigemura K, Yamamichi F, et al. International Comparison of Causative Bacteria and Antimicrobial Susceptibilities of Urinary Tract Infections between Kobe, Japan, and Surabaya, Indonesia. Jpn J Infect Dis 2018;71:8-13.
24. Purbowati R, Sugiharto, Listyawati AF, et al. Antibacterial and antibiofilm effect of silver and gold nanoparticles in Uropathogenic Escherichia coli. Berkala Penelitian Hayati 2021;27(2 SE-Articles).
25. Alhamid MZ, Hadi BS, Khumaeni A. Synthesis of silver nanoparticles using laser ablation method utilizing Nd:YAG laser. AIP Conf Proceed 2019;2202:20013.
26. Jailani A, Ahmed B, Lee JH, Lee J. Inhibition of Agrobacterium tumefaciens Growth and Biofilm Formation by Tannic Acid. Biomedicines 2022;10:1619.
27. Ibrahim H. Nanotechnology and Its Applications to Medicine: an over view. QJM Int J Med 2020;113:hcaa060.008.
28. Mutalik C, Lin IH, Krisnawati DI, et al. Antibacterial Pathways in Transition Metal-Based Nanocomposites: A Mechanistic Overview. Int J Nanomed 2022;17:6821-42.
29. Wang S, Gao Y, Jin Q, Ji J. Emerging antibacterial nanomedicine for enhanced antibiotic therapy. Biomat Sci 2020;8(:6825-39.
30. Garg P, Attri P, Sharma R, et al. Advances and Perspective on Antimicrobial Nanomaterials for Biomedical Applications. Front Nanotechnol 2022:4.
31. Balachandar R, Navaneethan R, Biruntha M, et al. Antibacterial activity of silver nanoparticles phytosynthesized from Glochidion candolleanum leaves. Materials Letters 2022;311:131572.
32. Alarjani KM, Huessien D, Rasheed RA, Kalaiyarasi M. Green synthesis of silver nanoparticles by Pisum sativum L. (pea) pod against multidrug resistant foodborne pathogens. J King Saud University - Sci 2022;34:101897.
33. Hashemi Z, Shirzadi-Ahodashti M, Mortazavi-Derazkola S, Ebrahimzadeh MA. Sustainable biosynthesis of metallic silver nanoparticles using barberry phenolic extract: Optimization and evaluation of photocatalytic, in vitro cytotoxicity, and antibacterial activities against multidrug-resistant bacteria. Inorg Chem Comm 2022;139.
34. Gouyau J, Duval RE, Boudier A, Lamouroux E. Investigation of Nanoparticle Metallic Core Antibacterial Activity: Gold and Silver Nanoparticles against Escherichia coli and Staphylococcus aureus. Int J Mol Sci 2021;22:1905.
35. Mobed A, Hasanzadeh M, Shadjou N, et al. Immobilization of ssDNA on the surface of silver nanoparticles-graphene quantum dots modified by gold nanoparticles towards biosensing of microorganism. Microchem J 2020;152:104286.
36. Miškovská A, Rabochová M, Michailidu J, et al. Antibiofilm activity of silver nanoparticles biosynthesized using viticultural waste. PLoS One 2022;17:e0272844.
37. Siddique MH, Aslam B, Imran M, et al. Effect of Silver Nanoparticles on Biofilm Formation and EPS Production of Multidrug-Resistant Klebsiella pneumoniae. BioMed Res Int 2020;2020:6398165.
38. Kong AS, Maran S, Yap PS, et al. Anti- and Pro-Oxidant Properties of Essential Oils against Antimicrobial Resistance. Antioxidants (Basel) 2022;11:1819.
39. De Silva C, Nawawi NM, Karim MMA, et al. The mechanistic action of biosynthesised silver nanoparticles and its application in aquaculture and livestock industries. Animals (Basel) 2021;11:2097.
40. Mathelié-Guinlet M, Asmar AT, Collet JF, Dufrêne YF. Lipoprotein Lpp regulates the mechanical properties of the E. coli cell envelope. Nature Comm 2020;11:1789.
41. Ali SG, Ansari MA, Alzohairy MA, et al. Biogenic Gold Nanoparticles as Potent Antibacterial and Antibiofilm Nano-Antibiotics against Pseudomonas aeruginosa. Antibiotics (Basel) 2020;9:100.
42. Gómez-Núñez MF, Castillo-López M, Sevilla-Castillo F, et al. Nanoparticle-Based Devices in the Control of Antibiotic Resistant Bacteria. Front Microbiol 2020;11:563821.
43. Ali S, Perveen S, Ali M, et al. Bioinspired morphology-controlled silver nanoparticles for antimicrobial application. Mat

Non-commercial use only

Sci Engin C 2020;108:110421.
44. Rodríguez-Serrano C, Guzmán-Moreno J, Ángeles-Chávez C, et al. Biosynthesis of silver nanoparticles by Fusarium scirpi and its potential as antimicrobial agent against uropathogenic Escherichia coli biofilms. PLoS One 2020;15:e0230275.
45. Funari R, Shen AQ. Detection and Characterization of Bacterial Biofilms and Biofilm-Based Sensors. ACS Sensors 2022;7:347-57.
46. Algabar FAA, Baqer BA. Detection of biofilm formation of (Serratia and E.coli) and determination of the inhibitory effect of Quercus plant extract against these infectious pathogens. Bionatura 2022;7:8.

Non-commercial use only