Prevention of nosocomial transmission and biofilm formation on novel biocompatible antimicrobial gloves impregnated with silver nanoparticles synthesized using *Eucalyptus citriodora* leaf extract

Supakit Paosen[1], Sakkarin Lethongkam[1], Suttiwan Wunnoo[1], Nussana Lehman[2], Ekwipoo Kalkornsurapranee[2], and Supayang Piyawan Voravuthikunchai[1*]

[1] Division of Biological Science, Faculty of Science, and Natural Product Research Center of Excellence, Excellence Research Laboratory on Natural Products, Prince of Songkla University, Songkhla, Thailand

[2] Division of Physical Science, Faculty of Science, Prince of Songkla University, Songkhla, Thailand

***Correspondence**:

Supayang Piyawan Voravuthikunchai

supayang.v@psu.ac.th

Abstract

Failure in the prevention of cross-transmission from contaminated gloves has been recognized as an important factor that contributes to the spread of several healthcare-associated infections. *Ex situ* coating process with silver nanoparticles (AgNPs) using *Eucalyptus citriodora* ethanolic leaf extract as reducing and capping agents to coat glove surfaces has been developed to prevent this mode of transmission. Elemental analysis of coated gloves showed 24.8 Wt% silver densely adhere on the glove surface. The coated gloves fully eradicated important hospital-acquired pathogens including Gram-positive bacteria, Gram-negative bacteria, and yeasts within 1 h. The coated gloves showed significant reduction, an average of 5 logs when tested against all standard strains and most clinical isolates ($p < 0.01$). Following prolonged exposure, the coating significantly reduced the numbers of most adhered pathogenic species, compared with uncoated gloves ($p < 0.0001$), which was observed by fluorescence microscopy. Scanning electron microscopy further confirmed that AgNPs coated-gloves reduced microbial adhesion of mixed-species biofilms, compared with uncoated gloves. A series of contamination and transmission assays demonstrated no transmission of viable organisms. Biocompatibility analysis confirmed high cell viability of HaCaT and L929 cells at all concentrations of AgNPs tested. The coated gloves were non-toxic with direct contact with L929 cells.

KEYWORDS

Silver nanoparticles, *Eucalyptus citriodora***,** gloves, healthcare-associated infections**,** cytotoxicity

1 | INTRODUCTION

A major concern for hospitals and other healthcare institutions remains the continuous threat of infections caused by a wide range of opportunistic microorganisms. Emerging pan-resistant Gram-negative pathogens, multidrug-resistant pathogens including methicillin-resistant *Staphylococcus aureus* and vancomycin-resistant enterococci have been identified as causative agents for healthcare-associated infections. Transmission of organisms by contact of gloves with surfaces following a contact with a pathogen source has been recognized as an important vector for the pathogenesis of healthcare-associated infections. World Health Organization has published guidelines for hand hygiene and gloving, which is a major focus of some public health and infection control programs (WHO, 2009). Pathogens are disseminated to the surrounding environment and contamination of surfaces in close contact to other patients can be incurred by overuse of gloves. Health care workers often fail to remove gloves between patients or between contacts with various sites on a single patient, and glove misuse is regularly present in all healthcare facilities worldwide, therefore facilitating the spread of microorganisms.

To avoid the scenarios of cross-contamination and transmission of pathogens through gloves onto surfaces, research efforts have been undertaken, in particular, towards antimicrobial modifications of gloves. Gloves coated with antimicrobial agents have been capable of preventing the spread of microorganisms. As examples, glove surfaces have been impregnated with brilliant green dye and chlorhexidine (Reitzel et al., 2009), or gloves have been coated with the active ingredient polyhexamethylene biguanide hydrochloride on the outside surface (Leitgeb et al., 2013). Surface modification involving fluorinated silica nanoparticles improved the protective ability against bacterial contamination from disposable gloves (Oh et al., 2016). However, while gloves bearing antimicrobial agents inhibited bacterial growth, there are several issues that still need to be overcome such as the

ineffectiveness against antimicrobial-resistant bacteria, and the long-term antimicrobial inefficiency.

In global attempts to reduce hazardous chemicals and materials, green chemistry is attractive in the science and medical fields. Silver nanoparticles (AgNPs) were synthesized by the green route using ethanolic leaf extract from *Eucalyptus citriodora* Hook for coating gloves. AgNPs exhibit a broad spectrum of activity against infectious pathogens associated with healthcare-associated infections (Paosen et al., 2017) with no observable toxic effects on human lung epithelial and red blood cells (Wintachai et al. 2019; Paosen et al. 2019). The highly efficacious AgNPs-coated gloves potentially provide an additional means of protection against the horizontal transmission of common pathogens in a hospital setting.

2 | MATERIALS AND METHODS

2.1 | Dip-coating of glove surfaces

Biosynthesis of silver nanoparticles (AgNPs) was done using *Eucalyptus citriodora* ethanolic leaf extract as described previously (Paosen et al., 2017). AgNPs was deposited on glove surfaces by dip-coating. Latex gloves were first cut into 2.5 cm× 2.5 cm pieces. Gloves were then dipped into the AgNPs suspension. The method involved dipping gloves for 10 min. AgNPs-coated gloves were then left to dry at room temperature for 24 h.

2.2 | Surface characterization

Analysis of surface morphology of uncoated and AgNPs-coated gloves was performed by field emission scanning electron microscope. The distribution and percentage coverage of AgNPs on glove surfaces were evaluated by energy dispersive x-ray spectroscopy.

2.3 | Microbial strains and growth conditions

Reference strains including *Acinetobacter baumannii* ATCC 19606, *Escherichia coli* ATCC 25922, *Pseudomonas aeruginosa* ATCC 10145, *Klebsiella pneumoniae* ATCC 700603, *Enterococcus faecalis* ATCC 29212, *Staphylococcus aureus* ATCC 25923, and *Candida*

albicans ATCC 90028 were used in this study. Clinical isolates (*A. baumannii* NPRCoE 160510, *E. coli* NPRCoE 161001, *K. pneumoniae* NPRCoE 160602, *P. aeruginosa* NPRCoE 160901, *E. faecalis* NPRCoE 160702, Methicillin-resistant *S. aureus* NPRCoE 160801, *C. albicans* NPRCoE 160120) taken from Songklanagarind hospital (Prince of Songkla University granted Ethical 121 approval to carry out the study within its facilities REC59-241-19-6). Bacterial and yeast strains were subcultured overnight at 37 °C on Mueller–Hinton agar (MHA) and Sabouraud dextrose agar (SDA), respectively.

2.4 | Disk diffusion assay

Microbial cell suspensions were adjusted to McFarland standard No. 0.5 and spread onto MHA for bacteria and SDA for fungi. AgNPs-coated gloves were placed on the plates and incubated overnight at 37 °C. Sizes of the observed inhibition zones were measured to estimate the diffusion from AgNPs-coated gloves.

2.5 | Antimicrobial efficacy testing

Effects of AgNPs coated gloves to prevent microbial transmission were evaluated for 1 h contact durations with microbial challenge inoculum. Testing was performed in triplicate for each challenge organism (separate AgNPs-coated glove for each test) following the method described in International Organization of Standardization 22196 for evaluating antimicrobial efficacy of antibacterial coating on plastic surfaces. Briefly, 2.5 cm films were cut from coated gloves and secured over the opening of a 50 ml centrifuge tube to ensure that the surface of the glove was flat, and then the surface was exposed to 1.0×10^6 colony-forming units (CFU)/ ml of different Gram-positive or Gram-negative bacteria, and yeast. A microscope cover slip was placed on top of the bacterial inoculums to ensure that a uniform thin film of inoculums would be evaluated. After exposure, all antimicrobial activity was halted by submersion in 10 ml of D/E Neutralizing Broth (Becton Dickinson, Sparks, MD). The subsequent culture was recovered and serially diluted for quantitative culturing. As a

comparator, uncoated control gloves were also tested (in triplicate) following 1-hour exposures to challenge inoculum, and the recovered viable organisms were quantified. By comparing the number of organisms recovered from the control gloves to the number of organisms recovered from the genuine antimicrobial gloves, a log 10 reduction produced by the AgNPs- coated gloves was calculated.

2.6 | Anti-biofilm formation

Uncoated and AgNPs-coated gloves were submerged in 10 ml of a bacterial suspension (1.0×10^6 CFU/ml) and incubated at 37 °C for 24 h. After incubation, the bacterial inoculum was removed, and samples were washed, placed in sterile saline, and sonicated for 15 min. The viable microbial cells in the suspensions were determined by 10-fold serial dilution and plated on agar. The plates were then incubated at 37 °C for 24 h and the colonies were counted.

2.7 | Antibiofilm activity assay by fluorescence microscopy image

S. aureus ATCC 25923, *P. aeruginosa* ATCC 27853, and *C. albicans* ATCC 90028 biofilms were expected to grow on glove samples according to the method mentioned above. After 24 h of incubation, the samples were removed and rinsed by saline solution. Biofilms on gloves were stained with LIVE/DEAD BacLight Bacterial Viability Kit (Sigma-Aldrich, USA) for 30 min in the dark followed by washing with PBS twice. The samples were observed at Nikon Eclipse 80i Advance Research Fluorescence microscope.

2.8 | Polymicrobial antibiofilm activity of AgNPs- coated gloves

The coated glove samples were immersed in mixed cultures of *S. aureus*, *P. aeruginosa*, and *C. albicans* and incubated at 37 °C for 24 h. Microbial biofilm morphology on the glove surfaces was observed under a scanning electron microscope (SEM).

2.9 | Pathogens contamination and transmission assay

The impact of AgNPs-coated gloves on the contamination of surfaces with *S. aureus*, *P. aeruginosa*, and *C. albicans*, and the subsequent pathogen transmission to other surfaces was evaluated using a method described previously (Reilman et al., 2015). Briefly, bacterial and yeast suspensions were adjusted to McFarland standard No. 0.5 (1.5×10^8 CFU/ml) and spread onto tryptic soy agar (TSA) and SDA, respectively. After inoculation, the plates were dried at 37 °C for approximately 30 min to allow bacteria to settle and to remove excess moisture. Contamination and transmission were achieved by pressing the gloved stamp (no.1) onto the plate inoculated with pathogens for approximately 10 sec (Figure 1a). The stamp was then used to contaminate a second stamp (no. 2) by pressing the two together for 5 sec (Figure 1b), after which it was pressed for 5 sec onto a clean TSA plate (Figure 1d). Subsequently, the second stamp was pressed against a third stamp (no. 3; Figure 1c), and both stamps were then pressed onto clean TSA plates for 5 sec (Figure 1d). All the plates were incubated overnight at 37 °C. Importantly, three non-coated control stamps were included and pressed onto different locations on the plates, to preclude a possible position-related assay bias.

2.10 | Cytotoxicity assay

The toxicity of the synthesized AgNPs was assessed using L929 murine fibroblast cell line and human keratinocytes HaCaT cells. The viability of cells after treatment was analyzed using the 3-[4,5-dimethylthiazole-2-yl]-2,5-diphenyltetrazolium bromide (MTT) assay. The fibroblast and keratinocyte cells were maintained in Dulbecco's modified eagle medium supplemented with 10% fetal bovine serum and antibiotics (100 U/ml penicillin, 100 µg/ml streptomycin) and incubated at 37 °C in a humidified 5% CO_2 atmosphere. The cells were trypsinized after reaching about 80% confluence. Approximately 4×10^4 cells/ml of cells were seeded in 96–well plates and incubated at 37 °C in a 5% CO_2 incubator. Following 24 h incubation, cell culture medium was removed and replaced DMEM medium containing AgNPs at concentrations of 0.02-1.43 µg/ml. The plates were incubated for further 24 h at 37

°C, 5% CO_2. The viability of cells was evaluated using 50 µg/mL of MTT and incubated for 4 h. Insoluble formazan crystals were dissolved with dimethyl sulfoxide and the absorbance was measured at 570 nm. Cell viability was calculated as follows,

$$\text{Cell viability (\%)} = \frac{\text{OD Treatment}}{\text{OD Control}} \times 100$$

2.11 | Evaluation of cytotoxicity based on the agar overlay test

The cytotoxicity of contact with glove materials was assessed using the agar overlay test following the method described in International Organization of Standardization 10993–5 biological evaluation of medical devices. Approximately 3×10^5 cells/ml of L292 cells were seeded in 96–well plates and incubated at 37 °C in a 5% CO_2 atmosphere for 24 h. Following incubation, the old medium was removed and the confluent cell monolayer was overlaid with 10 mL of 2% agar medium. Cells were stained with 0.01% neutral red vital dye (N-7005) after the agar had become solid. The plates were incubated for 20 min in the dark condition. The coated-glove materials (circular, diameter 5 mm) were placed on the agar layer and incubated at 37°C, 5% CO_2 for 24 h in the dark. Each test was carried out twice using at least three replicates for each experiment. The degree of cytotoxicity of the coated gloves was based on the size of the decolorized zone.

3 | RESULTS AND DISCUSSION

3.1 | Characterization and deposition of AgNPs on gloves

The structural and morphological properties of the AgNPs-coated gloves were analyzed using field emission scanning electron microscope. The AgNPs coated-gloves showed a uniform and very rough surface (Figure 2a). The roughness of the surfaces is difficult to attach to because the microorganism must stretch itself to wrap around the coating particles, which is an energy-expensive operation. Surface functionalization with AgNPs is capable of preventing bacterial attachment and biofilm formation. Coated medical devices have exhibited excellent antibacterial properties with a reduction in the surface attachment of

Staphylococcus aureus compared to the smooth surface (Cai et al., 2019). The surface constituents of the AgNPs-coated gloves include C, O, S, Si, Cl, N, and Ag as presented in Figure 2b. Elemental analysis of coated sutures showed Ag concentration of 24.8 Wt% (Figure 2b). The cytotoxicity of biosynthesized AgNPs against microorganisms may result from the oxidative dissolution of silver ions from the AgNPs embedded on the material surfaces. AgNPs interfere with bacterial membrane functions, and intracellular reactive oxygen species are generated by released silver ions (Yan et al., 2018). The antimicrobial properties of silver ions are directly proportional to their environmental concentration (Li et al., 2017).

3.2 | Antimicrobial activity of AgNPs-coated gloves

The antimicrobial activity of the AgNPs-coated gloves was assayed against common pathogens causing hospital-acquired infections including multidrug-resistant clinical isolates of Gram-negative bacteria, Gram-positive bacteria, and fungi (Table 1). The coated gloves could inhibit both reference strains and clinical isolates. Inhibition zones for Gram-negative bacteria, Gram-positive bacteria, and fungi ranged from 5.85-9.75, 10.05-11.50, and 7.15-7.53 mm, respectively.

3.3 | Evaluating antimicrobial efficacy of AgNPs-coated latex gloves following the method described in International Organization of Standardization 22196

Antimicrobial coating of medical devices, such that exhibits a rapid bactericidal effect, could be an ideal strategy to prevent medical device associated infections. The organisms tested are representative of common and emerging multidrug resistant (MDR) pathogens that account for the majority of hospital-acquired infections. This includes organisms such as methicillin-resistant *Stapphylococcus aureus*, which is considered to be endemic in many hospitals, and the emerging pan resistant (Turner et al., 2019) Gram-negative pathogen carbapenase-producing *Klebsiella pneumoniae*, which is quickly becoming a major healthcare threat

(Tumbarello et al., 2019). AgNPs-coated gloves killed a broad spectrum of Gram-positive bacteria, Gram-negative bacteria, and yeasts within 1 h (Figure 3). The coated gloves showed significant reduction, an average of 5 logs when tested against standard strains and most clinical isolates ($p < 0.01$). Two to three logs reduction were observed in *E. coli* NPRCoE 161001 and *K. pneumoniae* NPRCoE 160602 which still meet the acceptance criteria. A recent study has indicated that AgNPs exhibit activity against a wide range of pathogens. Silver ions bind irreversibly to the bacterial cell membranes and induce a reorganization of the membrane (Paosen et al., 2019). Mode of action of AgNPs is based on disrupting the bacterial cytoplasmic membrane causing leakage of macromolecular components (Ahmed et al., 2018).

3.4 | Microbial attachment to AgNPs-coated glove surfaces characterized by colony counts

During examinations, planktonic bacteria can adhere to glove surfaces and initiate biofilm formation. Dry surface biofilms containing *S. aureus* with the presence of self-produced extracellular polymeric substances immediately contaminated to gloves of healthcare worker which can be transferred to another surface. The efficacy of AgNPs-coated gloves with prolonged exposure to microorganisms completely killed most of the organisms within 24 h (Figure 4). The coating significantly reduced the numbers of most microorganisms adhering to the surfaces ($p < 0.0001$), *K. pneumoniae* ATCC 700603 and *P. aeruginosa* NPRCoE 160901 ($p < 0.001$), and *K. pneumoniae* NPRCoE 160602 ($p < 0.05$), when compared with uncoated gloves. Prolonged use of gloves, which might best protect healthcare workers from pathogenic threats, provides an ideal surface for pathogens to multiply on and thus increases the potential for breakthrough transmission and hospital-acquired infection. Results from this study indicate that AgNPs-coated gloves are highly efficacious in significantly reducing long-term pathogenic contamination. For instance, when glove surfaces come into contact with bacterial pathogens, bacterial adhesion occurs and the bacteria can subsequently form a

biofilm on these surfaces (Tahir et al., 2019). Bacterial biofilms are particularly difficult to eliminate using hygienic practices, such as physical washing and chemical treatments, because the bacteria are embedded in their own extracellular polymeric substances that guard against external physical forces and chemical agents (Corcoran et al., 2014). Glove surfaces have been modified in various ways to enhance their antibiofilm activity. Results for the efficacy of gloves impregnated with chlorhexidine and brilliant green with prolonged exposure to microorganisms exhibited a reduction of the pathogen (Reitzel et al., 2009). Another study developed antimicrobial gloves with fluorinated silica nanoparticles to improve the protective ability against bacterial attachment to surface gloves (Oh et al., 2016). The AgNPs have been shown to inhibit biofilm produced by pathogens through dissolving part of the extracellular matrix material and diffusing inside the biofilm matrix through the pores, reaching the cells in the deeper layers (Monteiro et al., 2015).

3.5 | Microscopic visualization of antibiofilm activity of AgNPs-coated gloves

Fluorescence microscope was applied to observe the distribution of live bacteria fluoresced green and dead bacteria fluoresced red in biofilms. The differences in the result of staining of microbial biofilms with uncoated gloves and AgNPs-coated gloves are presented in Figure 5. The results confirmed that AgNPs coated-gloves eliminated *S. aureus* biofilms. Fluorescence microscopy images were presented red-fluorescent DNA-specific stain that penetrates only cells with disrupted membranes and dead bacteria (Figure 5d). The images showed substantially lower abundance and viability of *P. aeruginosa* cells when grown on AgNPs-coated gloves (Figure 5e), compared with uncoated gloves (Figure 5b), suggesting that AgNPs killed and prevented microbial attachment.

3.6 | Polymicrobial inhibition of AgNPs-coated gloves

Microbial biofilms are notorious for expediting contamination of medical devices. Biofilms can be formed by either a single or multiple species of organisms. The antimicrobial coating

and surface alterations of medical devices provide promising opportunities in the prevention of biofilm formation on medical devices. Scanning electron microscopy was used to examine the surface morphology of the bacterial adhesion by the mixed culture of *P. aeruginosa*, *S. aureus***,** and *C. albicans* after 24 h of incubation. Polymicrobial cells became embedded and colonized in the uncoated gloves (Figure 6a), whereas small numbers of microbial cells were found on the coated surfaces (Figure 6b). The results demonstrate that AgNPs-coated gloves substantially prevented bacterial attachment. In general, Gram-negative bacterial species and *S. aureus* interacted in a competitive manner. The tested Gram-negative bacteria grew better in mixed dual-species biofilms than in their mono-species biofilms (Makovcova et al., 2017).

3.6 | AgNPs effectively prevent pathogen transmission

The most important route of transmission of hospital acquired infection pathogens is via contaminated hands of healthcare personnel. Improper glove disposal can contaminate floors and the surrounding environmental surfaces, and possible transmission can infect health care workers and other patients. The bacteriophage and chemical dye indicate highest environmental contamination nearest the health care workers (Munoz-Gutierrez et al., 2019). Touch transference assays were used to mimic contaminated conditions and subsequently transferred to healthcare workers' hands and other surfaces. Stamps covered with coated gloves were either coated with AgNPs or they were left untreated. These stamps were contaminated by pressing them on a medium inoculated with *Staphylococcus aureus* ATCC 25923, *Pseudomonas aeroginosa* ATCC 27853, and *Candida albicans* ATCC 90028. This assay demonstrated that for uncoated control stamps, the transmission of pathogens was detectable from the initially contaminated stamp to both the second and third stamps (Figure 7). AgNPs-coated stamps prevented transmission to stamps no. 2 and no. 3, and even stamp no. 1 did not transmit viable *S. aureus* (Figure 7a) and *C. albicans* (Figure 7b), suggesting that the antimicrobial coating was successful in controlling transmission. However, the imprint on the

bioassay plate was characterized by a large clearing zone. The number of transmitted *Pseudomonas aeruginosa* decreased visibly with each transfer (Figure 7c). Furthermore, the imprints left by the control stamps on the inoculated bioassay plate that was used for stamp contamination showed only the outlines of the stamps. Contamination of the first stamp was slightly reduced compared to the uncoated control stamps and, as expected, there was no inhibition of bacterial growth.

3.7 | MTT assay of cytotoxicity of AgNPs on human-derived keratinocyte HaCaT and L929 murine fibroblast cell line

Biosynthesized AgNPs have been proposed as effective antimicrobial agents against pathogens. Determination of cytotoxicity is important for effective clinical use. In the present study, L929 murine fibroblast (Figure 8a) and human keratinocyte HaCaT cells (Figure 8b) exposed to AgNPs at different concentrations (0.01–0.72 μg/ml) for 24 h showed viabilities of 98.83 ± 8.40% and 94.86 ± 5.50%, respectively. According to our previous report, 0.18 to 0.36 μg/ml of AgNPs were required to achieve minimum bactericidal concentration against important hospital-acquired pathogens, and hence, we used the maximum concentration of the AgNPs in the remainder of the assays (Paosen et al., 2019). The results of cell viability demonstrated that bactericidal concentrations showed no significant cytotoxicity towards the tested cells. AgNPs are prepared by various methods including physical, chemical and biological synthesis. Type of reaction agent use in the synthesis of AgNPs is a crucial factor determining cytotoxicity. Capping with a biocompatible material on the surface of AgNPs could remarkably reduce the cytotoxicity of AgNPs (Young et al., 2018). *Eucalyptus citriodora* is characterized as being harmless and environmentally friendly along with being a good reducing and stabilizing agent (Paosen et al., 2017). A previous study on toxicity of silver nanoparticles has evaluated cellular toxicity of AgNPs in fibroblast cells and reported that spherical AgNPs in the range 7–20 nm are non-toxic. The presence of AgNPs inside the

mitochondria could lead to alterations in reactive oxygen species production, and it seems that cellular antioxidant mechanisms in eukaryotic cells protect them from possible oxidative damage (Arora et al., 2009).

3.8 | Evaluation of cytotoxicity of AgNPs-coated gloves based on the agar overlay test

AgNPs used in various commercial products are mostly designed to come into direct contact with the skin. In this study, we investigated the effects of AgNPs-coated gloves on the skin using the L929 murine fibroblast cell line model. The International Standards Organization recommends the use of established fibroblast cells in cytotoxicity tests, and conclusions regarding the possible toxicity *in vivo* are limited (ISO 10993-5). The cells were examined under light microscope and the cytotoxicity of the coated gloves was determined by lysis of the cells adjacent to the gloves and decolorization of the stained cells. The decolorization zones of the experimental materials are presented in Figure 9. This result indicates that the AgNPs-coated gloves pose negligible cytotoxicity to the fibroblast cells. In a recent study our group biosynthesized AgNPs using Eucalyptus extract and incorporated them into materials. The coated medical devices provided broad-spectrum activity against pathogens and prolonged antimicrobial activity with no cytotoxic effects against human lung epithelial cells (Lethongkam et al., 2020, Daengngam et al., 2019). *Ex situ* AgNPs-coated surgical sutures with biosynthesized AgNPs did not significantly affect the viability of HaCaT keratinocyte cells (Syukri et al., 2020). Films containing AgNPs synthesized using eucalyptus leaf extract were biocompatible with human epithelial cell line Caco-2 (Nwabor et al., 2020).

4 | CONCLUSIONS

In conclusion, the present study demonstrated the feasibility of AgNPs-coated gloves that can be regarded as antimicrobial gloves. Based on the results, the coated gloves exhibited effective antimicrobial activity, especially against multidrug-resistant pathogens. The highly

efficacious AgNPs-coated gloves may be appropriate for protecting against or reducing cross-contamination and indirect transmission of pathogens in the hospital setting.

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CONFLICTS OF INTEREST

There are no conflicts of interest to declare.

AUTHOR CONTRIBUTIONS

Supakit Paosen: study conception and design, analysis and interpretation of data, drafting of the manuscript. Sakkarin Lethongkam, Suttiwan Wunnoo, Nussana Lehman and Ekwipoo Kalkornsurapranee: study conception and design, drafting of the manuscript. Supayang Piyawan Voravuthikunchai: funding acquisition, supervision, study conception, interpretation of data, critical revision.

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TABLE 1 Antimicrobial activity of AgNPs-coated gloves was determined by agar disk diffusion method

Pathogenic organisms	**Zone of diameter (mm)**	
	Uncoated gloves	**AgNPs-coated glove**
Reference Strains		
A. baumannii ATCC 19606	6.20±0.85	9.75±0.35
E. coli ATCC 25922	6.05±0.07	5.85±1.20
K. pneumoniae ATCC 700603	6.10±0.14	6.55±0.64
A. baumannii NPRCoE 160510	-[a]	7.30±0.00
P. aeruginosa ATCC 27853	-[a]	7.15±0.49
E. faecalis ATCC 29212	-[a]	10.40±0.99
S. aureus ATCC 25923	6.15±0.21	10.05±0.64
C. albicans ATCC 90028	-[a]	7.53±0.11
Clinical isolates		
E. coli NPRCoE 161001	6.10±0.14	8.50±1.41
K. pneumoniae NPRCoE 160602	6.30±0.28	6.90±0.42
P. aeruginosa NPRCoE 160901	-[a]	5.85±0.21
E. faecalis NPRCoE 160702	6.05±0.07	11.50±0.71
Methicillin-resistant *S. aureus* NPRCoE 160801	6.30±0.42	11.60±2.26
C. albicans NPRCoE 160120	-[a]	7.15±0.21

[a]no zone of inhibition

FIGURE LEGEND

FIGURE 1 Contamination procedure; a first stamp (no. 1) was pressed for 10 sec onto a tryptic soy agar (TSA) plate inoculated with microorganisms (a). First transmission step; stamp no. 1 was pressed to stamp no. 2 for 5 sec (b). Second transmission step; stamp no. 2 was pressed to stamp no. 3 for 5 sec (c). Contamination of stamps with pathogens was assessed by pressing the stamps onto TSA plates (d).

FIGURE 2 The distribution of AgNPs-coated gloves by energy dispersive x-ray (a). Field emission scanning electron microscope images of braided AgNPs-coated gloves (b).

FIGURE 3 Antimicrobial efficacy of AgNPs-coated gloves tested for 1-h exposure to reference strains (a) and multidrug-resistant clinical organisms (b) following the method described in International Organization of Standardization 22196. The acceptance criteria will be log reduction ≥ 2. All data were obtained from triplicate samples and values were reported as mean ± SD. The different letters indicate a statistically significant difference between uncoated gloves and AgNPs-coated gloves (*$p < 0.05$, ** $p < 0.01$).

FIGURE 4 Long-term exposure. Mean colony counts were recorded for AgNPs-coated gloves after 24- h exposure to reference strains (a) and multidrug-resistant clinical organisms (b). The data were obtained by the pour plating method. The different letters indicate a statistically significant difference between uncoated gloves and AgNPs-coated gloves (*$p < 0.05$, ** $p < 0.01$*** $p < 0.001$, ****$p < 0.0001$).

FIGURE 5 Fluorescence microscopy images of *S. aureus* ATCC 25923 (a, d), *P. aeruginosa* ATCC 27853 (b, e), and *C. albicans* ATCC 90028 (e, f) biofilms incubated with uncoated gloves (a-c) and AgNPs-coated gloves (d-f) for 24 h. Microorganisms were stained with LIVE/DEAD BacLight Bacterial Viability Kit. Cells with intact membranes were stained fluorescent green with SYTO9, whereas cells with damaged membranes were stained fluorescent red with propidium iodide.

FIGURE 6 Scanning electron microscopic (SEM) analysis. SEM micrograph of polymicrobial anti biofilm activity of uncoated gloves (a, b) and AgNPs- coated gloves (c, d). Microbial cells on glove surfaces (as indicated by the red arrow) were observed at the magnification of × 5000 and × 10,000.

FIGURE 7 Controls for the contamination and transmission assay. The first columns show the imprint that was left on the contamination plate (inoculated with *S. aureus* ATCC 25923 (a), *P. aeruginosa* ATCC 27853 (b), and *C. albicans* ATCC 90028 (c) after overnight incubation. The first columns depict the contamination of the three stamps with pathogens as reflected by

colony formation on the fresh TSA plates onto which the AgNPs-coated stamps were pressed. Representative images are shown after experiments were performed in triplicate.

FIGURE 8 The percentage of viable of L929 murine fibroblast (a) and human keratinocyte HaCaT cells (b) after incubation with AgNPs at different concentrations for 24 h. All data were obtained from triplicate samples and values were reported as mean ± SD.

FIGURE 9 Evaluation of cytotoxicity based on the agar overlay test following the method described in International Organization of Standardization 10993-5. Representative images are shown after experiments were performed in triplicate.

FIGURE 1

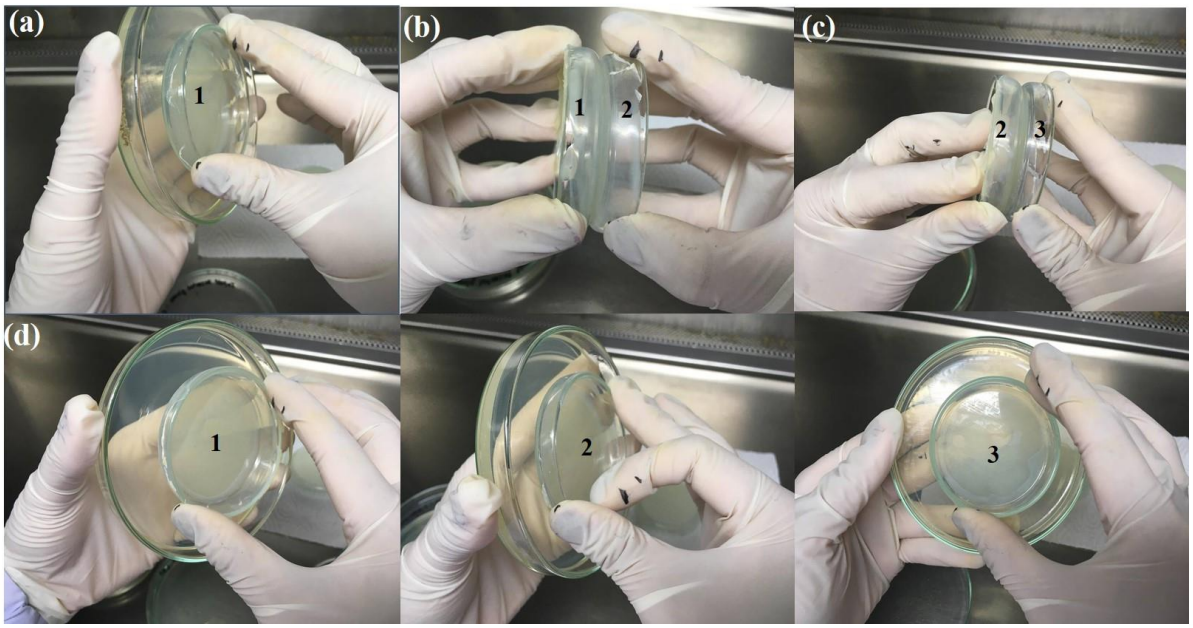


FIGURE 2

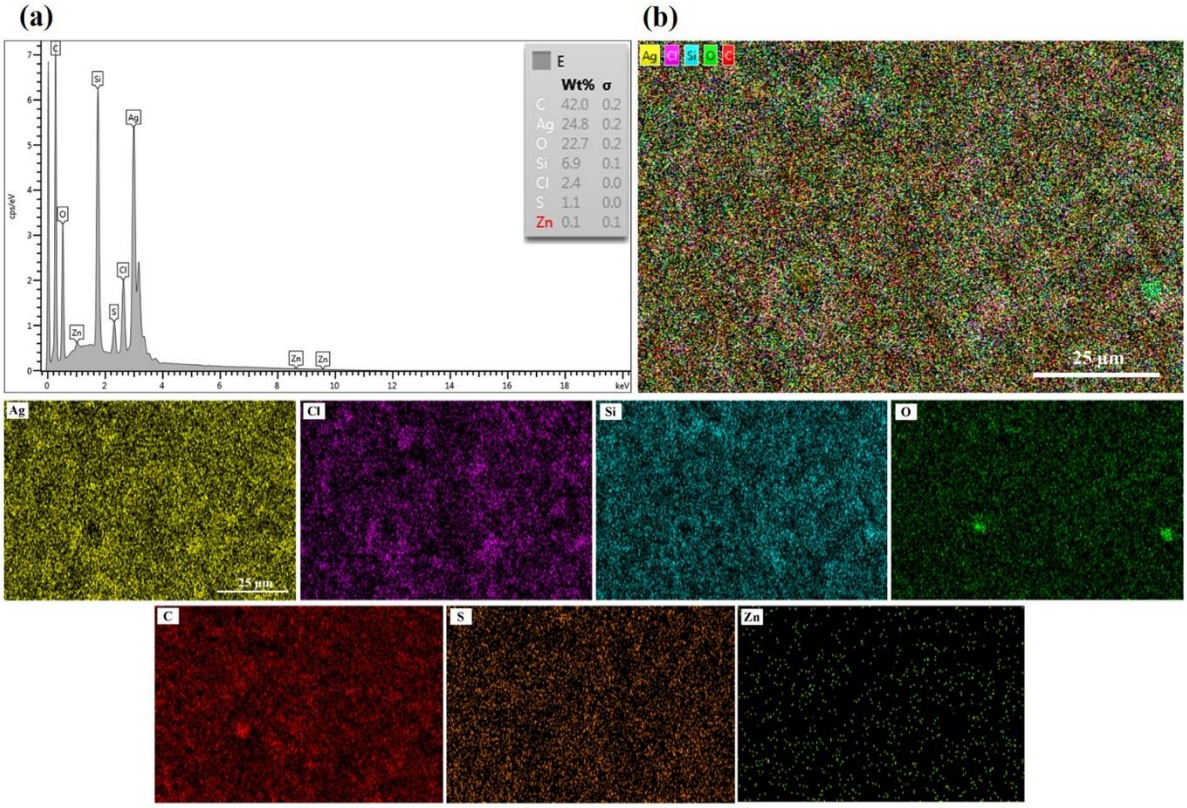

(a)
(b)
E
Wt% σ
C 42.0 0.2
Ag 24.8 0.2
O 22.7 0.2
Si 6.9 0.1
Cl 2.4 0.0
S 1.1 0.0
Zn 0.1 0.1
cps/eV
keV
25 µm
Ag
Cl
Si
O
C
S
Zn

FIGURE 3

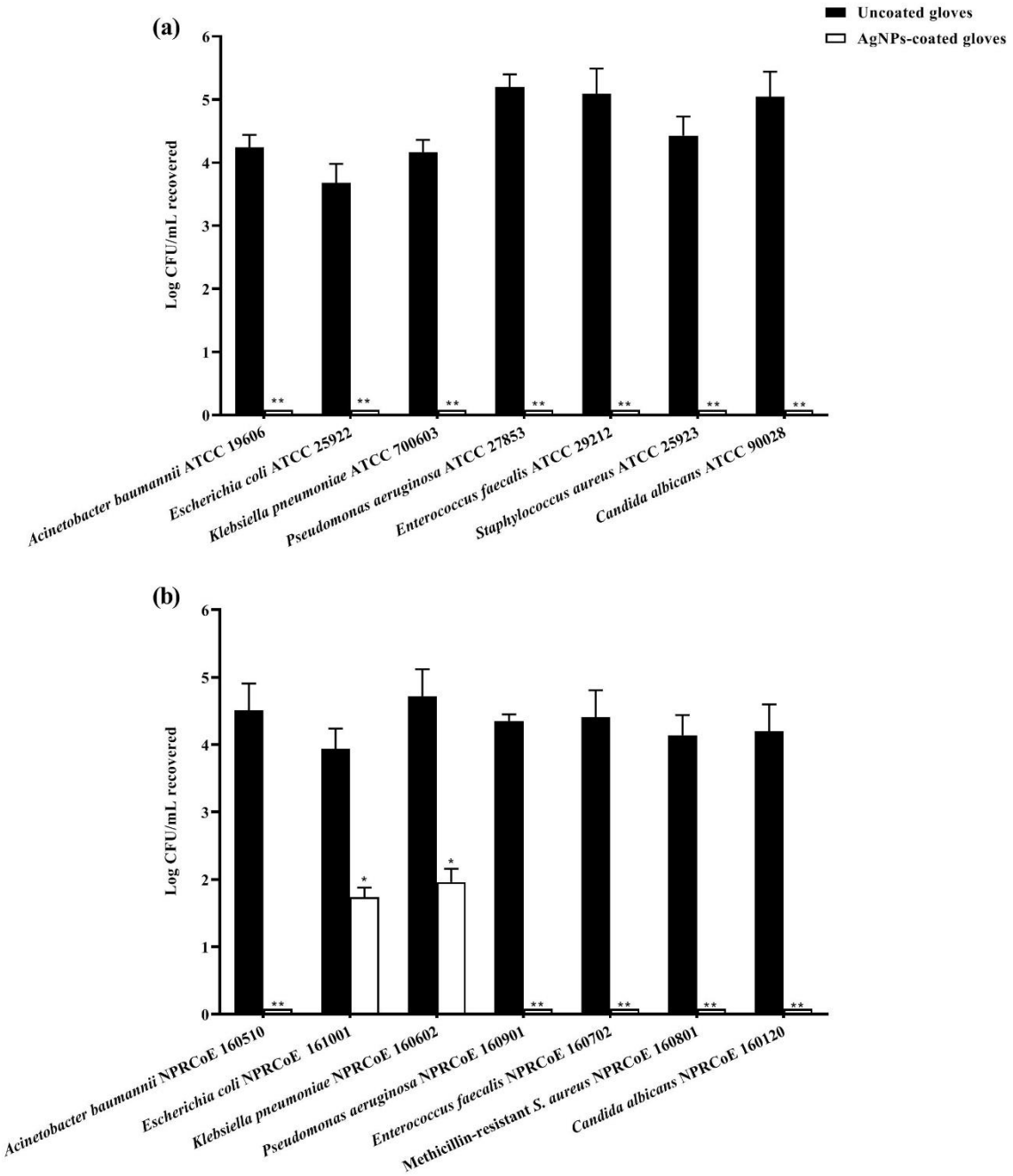

(a)
Uncoated gloves
AgNPs-coated gloves
Log CFU/mL recovered
0
1
2
3
4
5
6
**
Acinetobacter baumannii ATCC 19606
Escherichia coli ATCC 25922
Klebsiella pneumoniae ATCC 700603
Pseudomonas aeruginosa ATCC 27853
Enterococcus faecalis ATCC 29212
Staphylococcus aureus ATCC 25923
Candida albicans ATCC 90028
(b)
Log CFU/mL recovered
0
1
2
3
4
5
6
*
**
Acinetobacter baumannii NPRCoE 160510
Escherichia coli NPRCoE 161001
Klebsiella pneumoniae NPRCoE 160602
Pseudomonas aeruginosa NPRCoE 160901
Enterococcus faecalis NPRCoE 160702
Methicillin-resistant S. aureus NPRCoE 160801
Candida albicans NPRCoE 160120

FIGURE 4

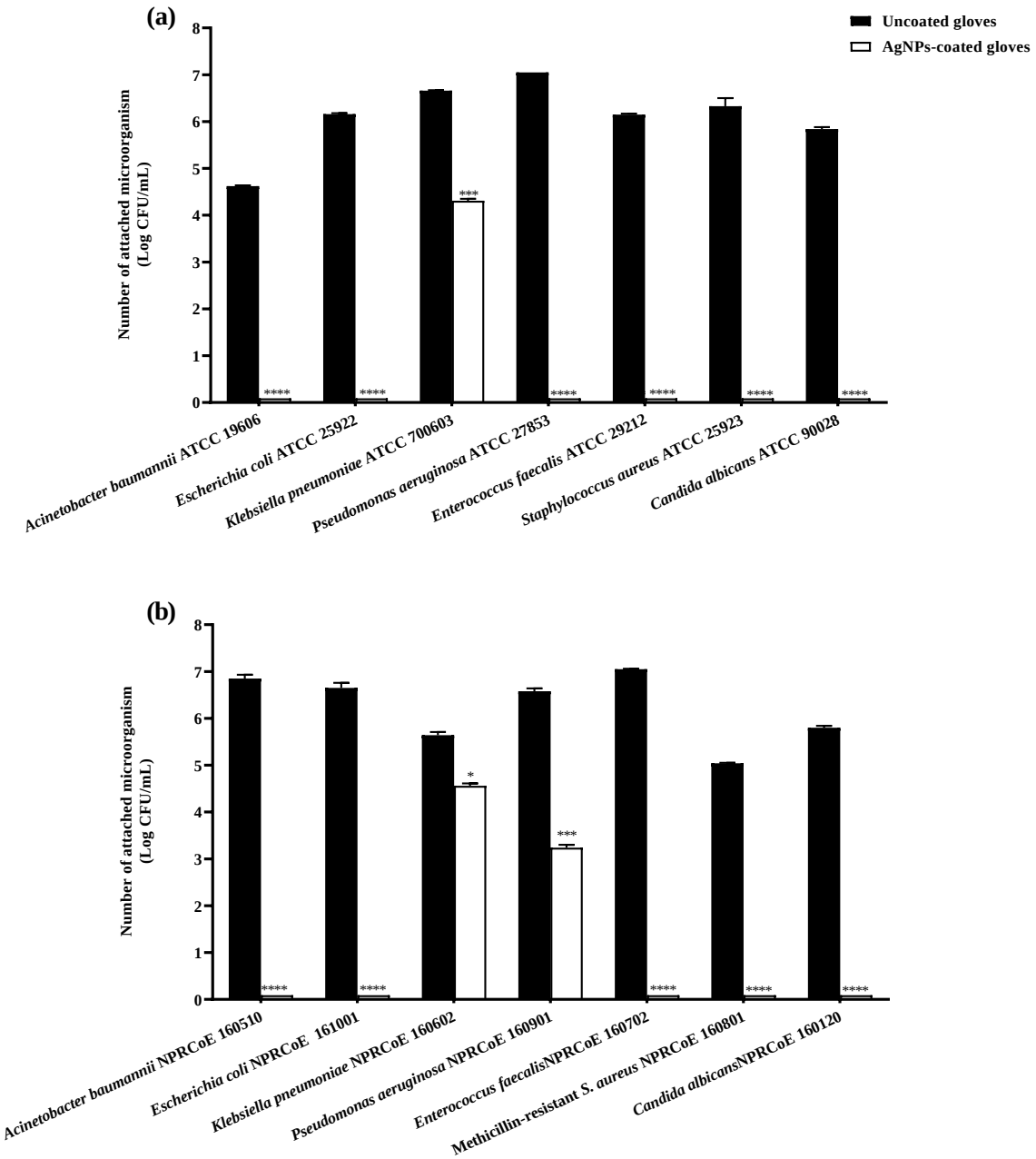

(a)
Uncoated gloves
AgNPs-coated gloves
Number of attached microorganism (Log CFU/mL)
0
1
2
3
4
5
6
7
8
***
****
Acinetobacter baumannii ATCC 19606
Escherichia coli ATCC 25922
Klebsiella pneumoniae ATCC 700603
Pseudomonas aeruginosa ATCC 27853
Enterococcus faecalis ATCC 29212
Staphylococcus aureus ATCC 25923
Candida albicans ATCC 90028
(b)
Number of attached microorganism (Log CFU/mL)
0
1
2
3
4
5
6
7
8
*
***
****
Acinetobacter baumannii NPRCoE 160510
Escherichia coli NPRCoE 161001
Klebsiella pneumoniae NPRCoE 160602
Pseudomonas aeruginosa NPRCoE 160901
Enterococcus faecalis NPRCoE 160702
Methicillin-resistant S. aureus NPRCoE 160801
Candida albicans NPRCoE 160120

FIGURE 5

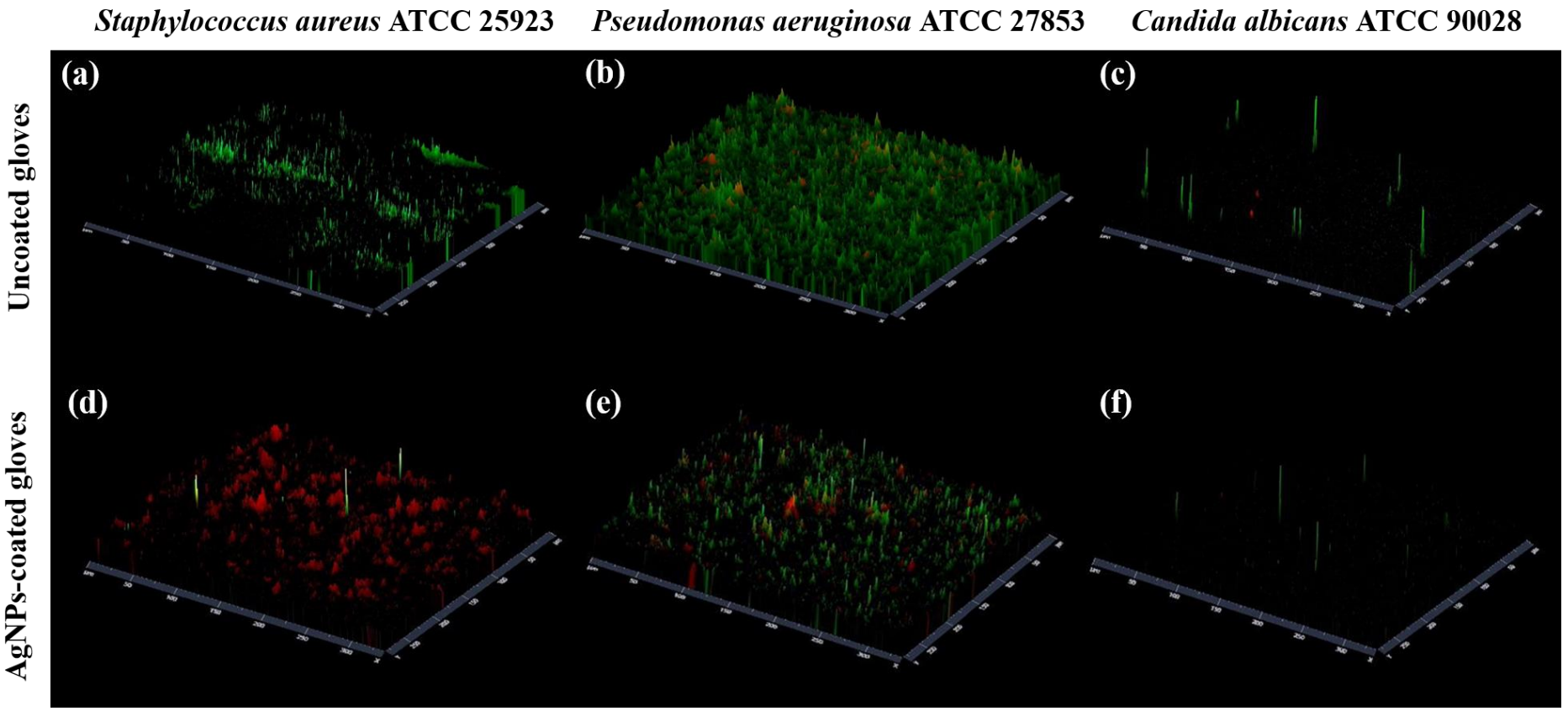

Staphylococcus aureus ATCC 25923
Pseudomonas aeruginosa ATCC 27853
Candida albicans ATCC 90028
Uncoated gloves
AgNPs-coated gloves
(a)
(b)
(c)
(d)
(e)
(f)

FIGURE 6

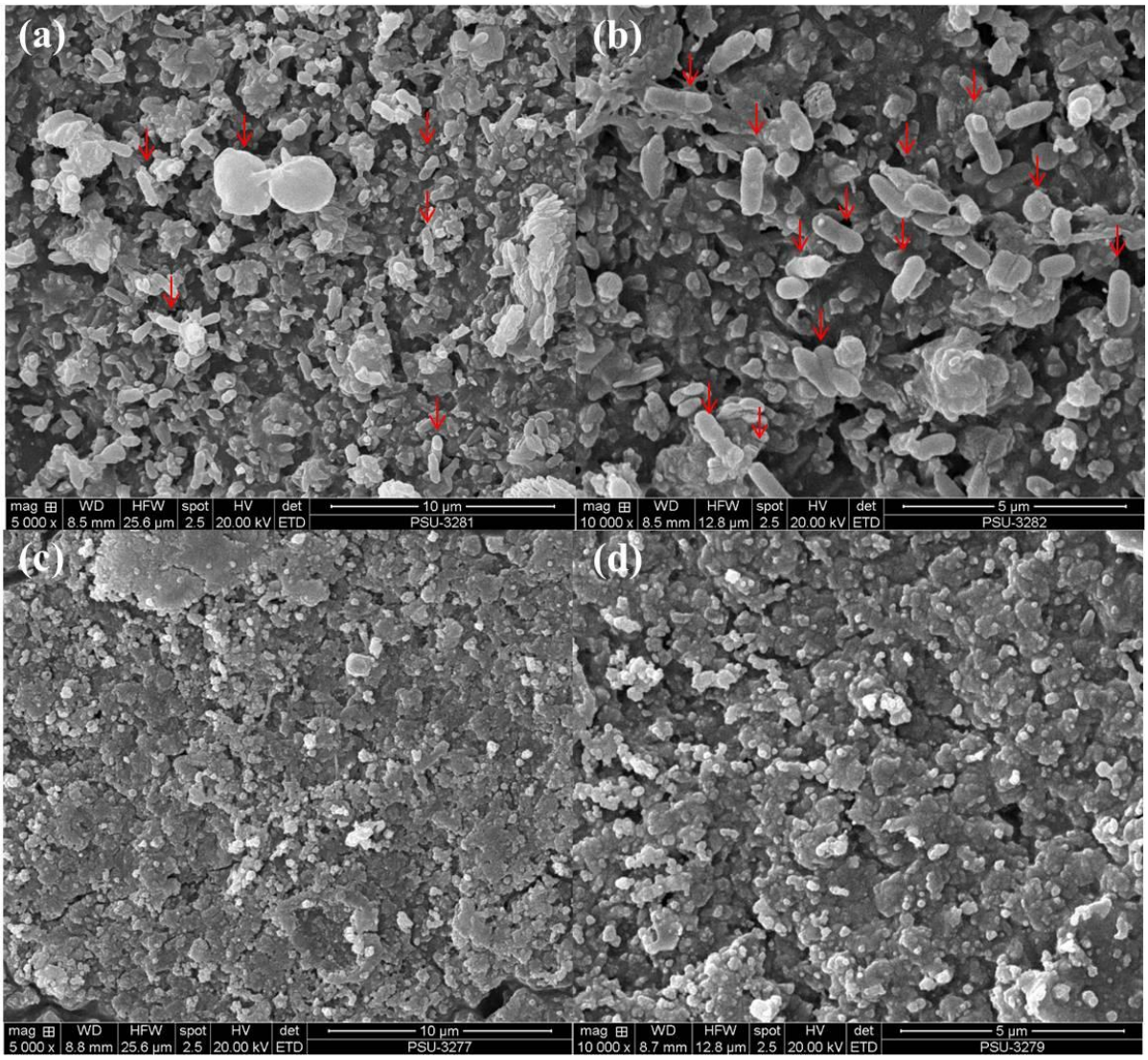

(a)
mag WD HFW spot HV det
5 000 x 8.5 mm 25.6 µm 2.5 20.00 kV ETD
10 µm
PSU-3281
(b)
mag WD HFW spot HV det
10 000 x 8.5 mm 12.8 µm 2.5 20.00 kV ETD
5 µm
PSU-3282
(c)
mag WD HFW spot HV det
5 000 x 8.8 mm 25.6 µm 2.5 20.00 kV ETD
10 µm
PSU-3277
(d)
mag WD HFW spot HV det
10 000 x 8.7 mm 12.8 µm 2.5 20.00 kV ETD
5 µm
PSU-3279

FIGURE 7

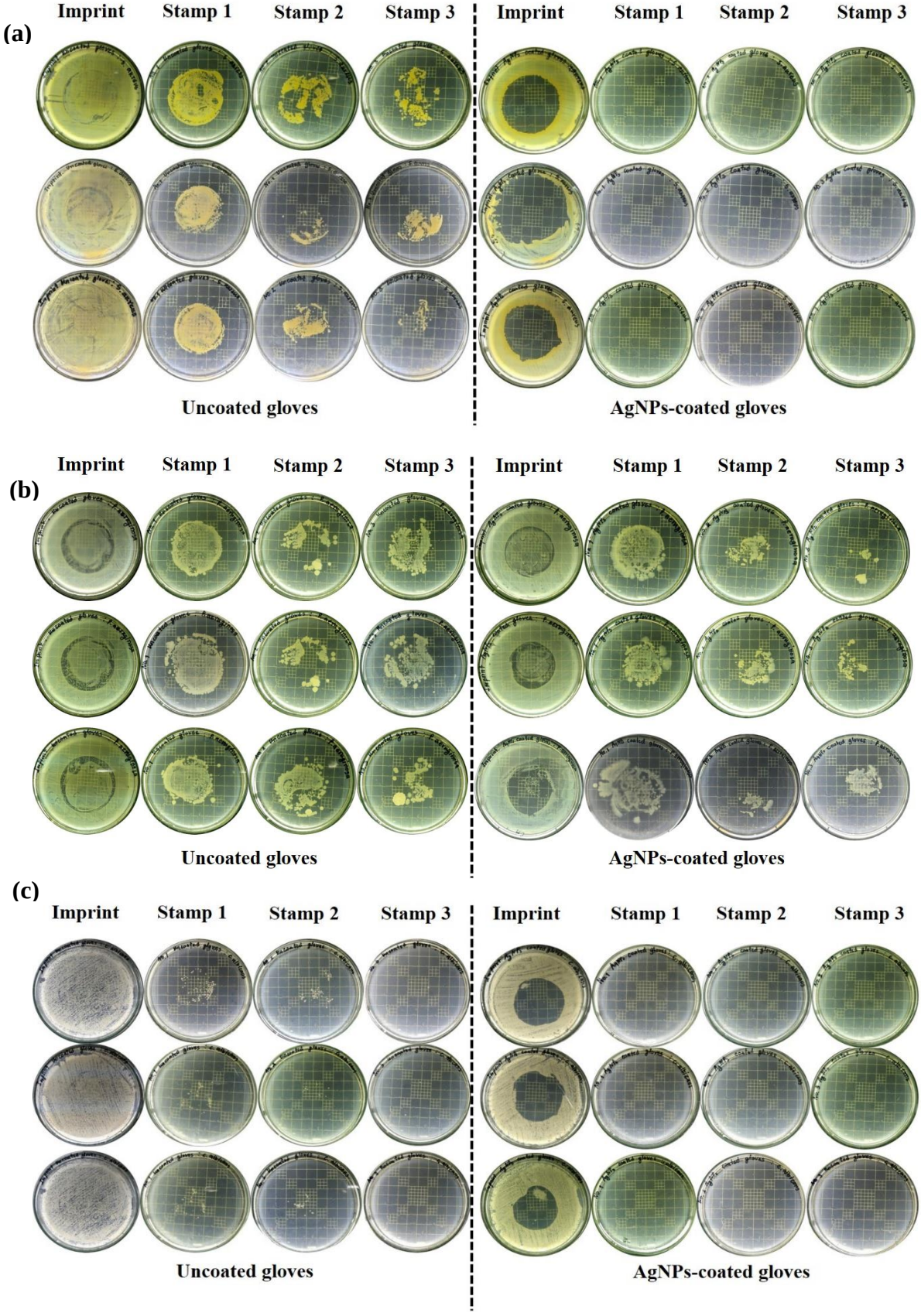

(a)
Imprint
Stamp 1
Stamp 2
Stamp 3
Imprint
Stamp 1
Stamp 2
Stamp 3
Uncoated gloves
AgNPs-coated gloves
(b)
Imprint
Stamp 1
Stamp 2
Stamp 3
Imprint
Stamp 1
Stamp 2
Stamp 3
Uncoated gloves
AgNPs-coated gloves
(c)
Imprint
Stamp 1
Stamp 2
Stamp 3
Imprint
Stamp 1
Stamp 2
Stamp 3
Uncoated gloves
AgNPs-coated gloves

FIGURE 8

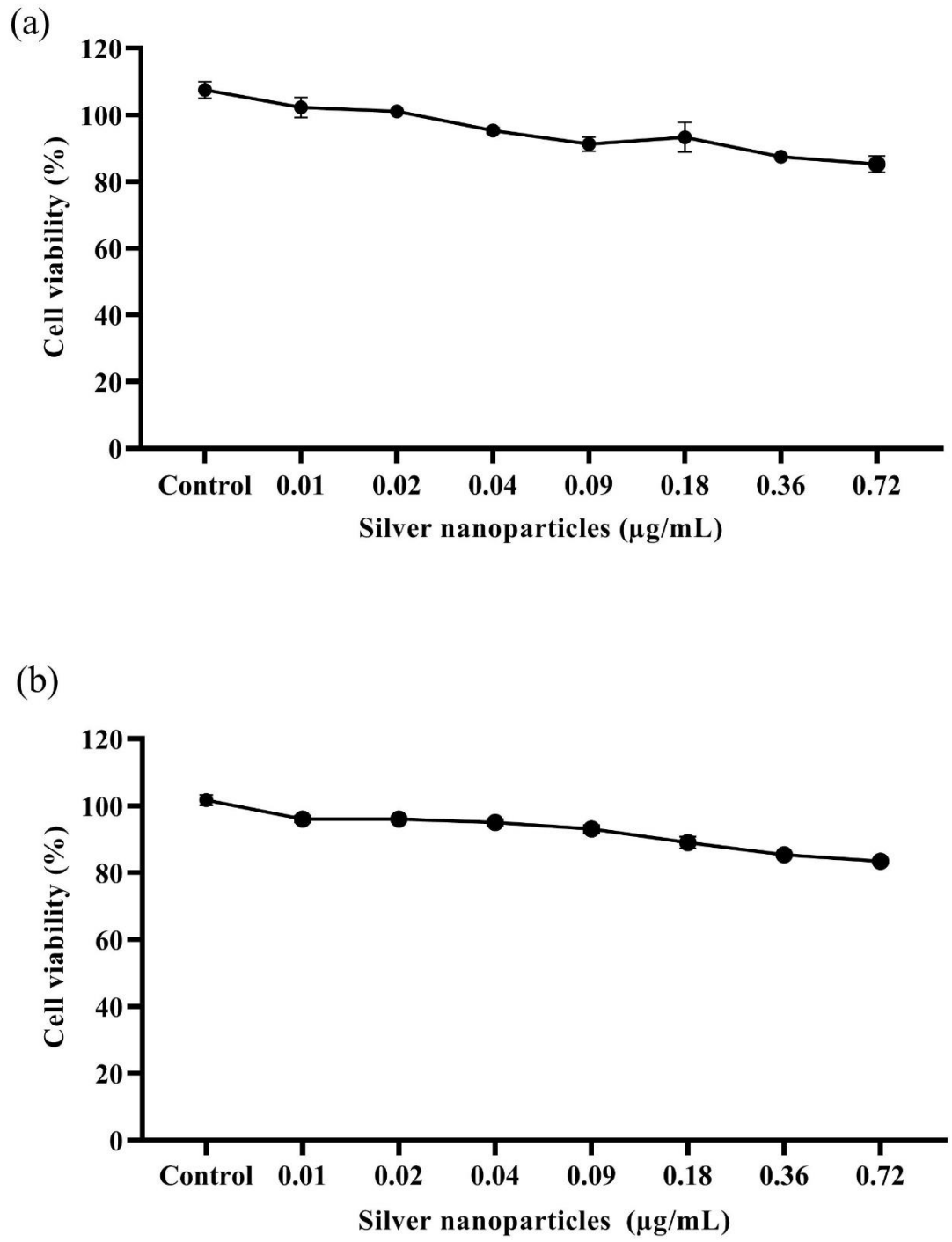
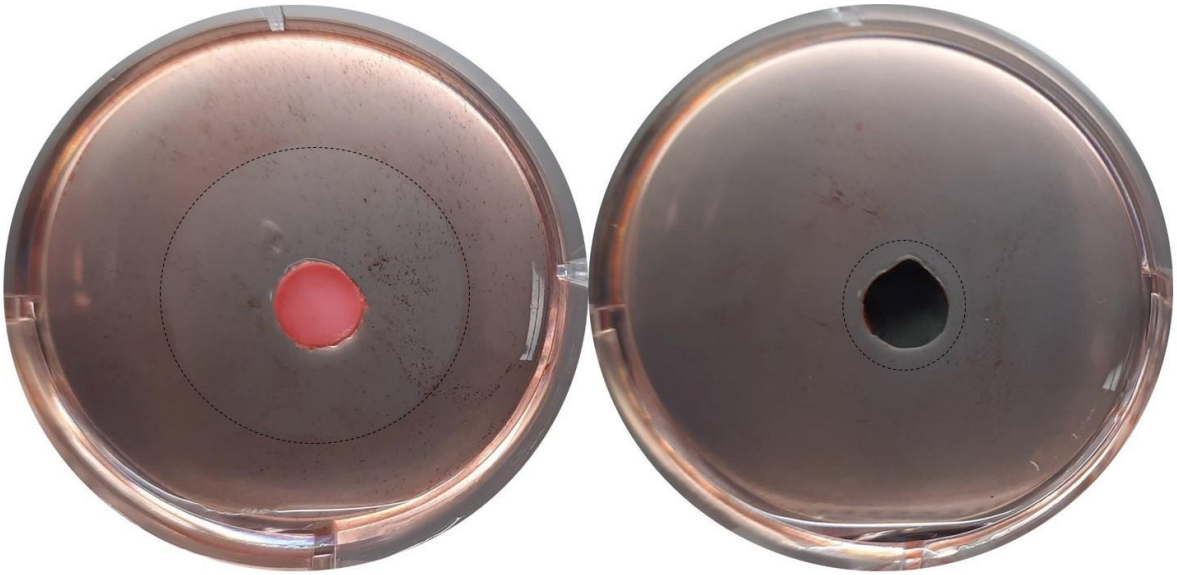

(a)
120
100
80
60
40
20
0
Cell viability (%)
Control
0.01
0.02
0.04
0.09
0.18
0.36
0.72
Silver nanoparticles (µg/mL)
(b)
120
100
80
60
40
20
0
Cell viability (%)
Control
0.01
0.02
0.04
0.09
0.18
0.36
0.72
Silver nanoparticles (µg/mL)

FIGURE 9



Positive control **AgNPs-coated gloves**