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Silver nanoparticles as potential antiviral agents against African swine fever virus

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Tran Thi Ngoc Dung[1,5], **Vu Nang Nam**[1], **Tran Thi Nhan**[1], **Trinh Thi Bich Ngoc**[2], **Luu Quang Minh**[3], **Bui Thi To Nga**[2], **Van Phan Le**[2,5] and **Dang Viet Quang**[4,5]

[1] Institute of Environmental Technology, Vietnam Academy of Science and Technology, 18 Hoang Quoc Viet, Hanoi, Vietnam
[2] College of Veterinary Medicine, Vietnam National University of Agriculture, Hanoi, Vietnam
[3] Ministry of Science and Technology (MOST), Vietnam
[4] Faculty of Biotechnology, Chemistry and Environmental Engineering, Phenikaa University, Hanoi 12116, Vietnam
[5] Authors to whom any correspondence should be addressed.

E-mail: ttndzung@gmail.com, ttndzung@ietvn.vn, letranphan@vnua.edu.vn and quang.dangviet@phenikaa-uni.edu.vn

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Abstract

African swine fever virus (ASFV) is the cause of a highly contagious and fatal disease in domestic swine, but no vaccine or effective medicine is available so far, therefore, looking for a novel and effective anti-ASFV agent is a very imperative mission. Silver nanoparticles (SNPs) have been recently emerged as novel antiviral agents against numerous viruses, but, their antiviral activity against ASFV has not been investigated. In this study, the antiviral ability of SNPs against ASFV was reported. The microbial contamination in the pig house was significantly reduced by spraying the SNP solution 25 ppm. SNP solution with the concentration of 0.78 ppm does not show any toxicity to porcine alveolar macrophage cells; while completely inhibits ASFV at the titer of 10^3 HAD_{50}. This study confirms that SNPs have a highly antiviral ability against ASFV and is a promising disinfectant that can be used to prevent the ASFV transmission.

1. Introduction

African swine fever virus (ASFV) is a member of the *Asfivirus* genus within the *Asfarviridae* family, a double-stranded DNA virus with a complex structure in an icosahedral morphology [1]. ASFV causes a viral disease of swine with a very high mortality in domestic pigs, while it is asymptomatic in the natural suid reservoir hosts. The virus can be transmitted via direct contact with infected animals, by the bites of infected arthropods, particularly soft ticks of the *Ornithodoros* genus, and via contact with materials or object contaminated with virus such as uncooked meat, blood or fluid from infected pigs [2–4]. The virus is persistent in blood and tissues after death make it easily to be transmitted via pork product transportation. The virus can be found in all secretions and excretions, particularly in the oronasal fluid. Airborne transmission also occurs in a pig house [3]. The outbreak of the disease may cause a significant economic consequence for the affected region, vaccination is the best control measure, but, unfortunately, there is no vaccine commercially available so far. The vaccine development has been deterred by large gaps in the understanding of ASFV infection, immunity, and the mechanism by which the virus modulates the host response to infection [5]. Without a vaccine against ASFV, early diagnosis and effective sanitary measures are very important strategies to eliminate the disease in the affected area.

Since there is no vaccine available, the implementation of biosecurity measures is still the key for the disease control and prevention. Besides the strict regulations for animal and animal product transportation, the pork-related waste management, and the infected area surveillance, an effective disinfection measure plays a very important role in controlling the disease spread [6–8]. ASFV is inactivated by pH $<$ 3.9 or $>$11.5 and some traditional disinfectants as calcium hydroxide, hypochlorite, formalin, ortho-phenylphenol, Glutaraldehyde and iodine compounds [6, 9]. The traditional disinfectants usually pose some disadvantages such as bad smell,

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quick loss of antiviral activity, and carcinogenic potential; therefore, only several disinfectants are recommended by the Environmental Protection Agency in the United States of the use in the combat against ASFV [9].

Recently, significant effort has been spent to find more effective antiviral agents, particularly the naturally originated ones, in the hope to develop an effective drug to treat the disease or antiviral agent for disinfection [5, 10, 11]. Resveratrol and Oxyresveratrol produced by plants (found in skin of grape, blueberries, raspberries, and peanuts) in response to biotic and abiotic stress can inhibit viral infection by disrupting cellular functions [1]. Genistein exhibits the most potent anti-ASFV-Ba71V activity with 3.8 log reduction in the viral titer at a concentration of 50 mM and 8 hpi [12]. The antiviral activity of Genistain on ASFV Ba71V strain is determined by several ways including impairing the viral DNA synthesis, inhibiting post-entry stages of ASFV life cycle, and inhibiting the activity of ASFV type II topoisomerase enzyme. The sulfated polysaccharides can inhibit the virus adsorption on host cell [13]. Apigenin, a natural flavonoid found in many plants can inhibit ASFV-specific protein synthesis and viral factory formation inducing 99.99% ASFV yield reduction by adding at 1 h post-inoculation [14]. Fluoroquinolones was found to impact on the viral DNA replication and ASFV protein synthesis that disrupting the viral infection [15]. A class of HDAC inhibitors can abolish ASFV-Ba71V replication and the late protein synthesis [16]. Even though, extensive attempt has been spent, no effective drug is commercially available for disease treatment; therefore, it is still a challenge to find effective disinfectants with highly antiviral ability.

Silver nanoparticles (SNPs) have been widely used for as a disinfectant in various applications from daily hygiene to medication [17–21]. Recently, nanomaterials including silver nanoparticles have emerged as a novel class of therapeutic agents that can inhibit the viral replication [22–24]. It is believed that SNPs, particularly particles with diameters below 10 nm, can interact with active sulfur groups in gp120 glycoprotein knobs inducing the inhibition of the of human immunodeficiency virus (HIV-1) infection [25–27] and a similar interaction could be found in herpes simplex virus type 1 (HVS-1) [28], H1N1 influenza A [29–31], and H3N2 influenza viruses [32]. SNPs are more potent than gold nanoparticles, but do not show acute cytotoxicity for Hut/CCR5 and human peripheral blood monocular cells, while inhibit HIV-1 replication [33]. The stabilizer used for SNPs synthesis also influences on their antiviral activity; a higher antiviral activity against RSV can be received when SNPs stabilized by curcumin in compared with citric acid [34]. Following the HIV-1, it has been demonstrated that SNPs can interact and inhibit many other types of viruses which cause human viral diseases such as HVS-1 and 2 [28, 35], hepatitis B [36], respiratory syncytial virus (RSV) [37], H1N1 influenza A [29, 31], and H3N2 influenza [32], human parainfluenza type 3 [38], poliovirus [39], and adenovirus type 3 [40]. Several studies have investigated the antiviral activity of SNPs against viral disease in animals and indicated that SNPs can effectively inhibit Tacaribe virus in bats [41], infectious bursal disease virus caused gumboro disease in chicken [42] and transmissible gastroenteritis virus (TGEV) that can cause severe diarrhea in pigs [43]. A SNP-modified grapheme oxide also proved highly antiviral ability against porcine reproductive and respiratory syndrome virus (PRRSV) known as blue-ear pig disease and porcine epidemic diarrhea virus (PEDV) [44].

The widespread of ASFV disease occurred in Europe, China, and several other countries causes a great economic loss and threat to the worldwide food security [45–52], however, the disease control and prevention are still based on the biosecurity measures due to the lack of vaccine and treatment drug. Thus, a demand for an effective antiviral agent to fight against the disease widespread is very high and imperative. SNPs have posed highly antiviral activity on numerous viruses, but, their antiviral ability against ASFV has not been investigated. Therefore, in this study, the antiviral activity of SNPs against ASFV will be examined to evaluate their potential use in the control of ASFV disease transmission.

2. Experimental

2.1. Materials and ethic statement

Sodium RPMI 1640 (1X), Anti-Anti (100X) antibiotic, and Fetal bovine serum were provided by Gibco. BHI, NA and plate count agar medium were purchased from Sigma Aldrich. Silver nitrate ($AgNO_3$, purity $>$ 99 %) and sodium borohydride ($NaBH_4$, 99%) were received from Merck. Acetic acid in practical grade purity was obtained from Canto Chemical. β-chitosan was provided by a local supplier. All experiments with live ASFV were carried out in a Key laboratory for veterinary biotechnology, Vietnam National University of Agriculture. This study complied with the ARRIVE guidelines and was conducted in strict accordance with the U K animals (scientific procedures) Act, 1986 and associated guidelines, EU Directive 2010/63/EU for animal experiments.

2.2. Silver nanoparticle synthesis

SNPs were synthesized by a chemical reduction method as described elsewhere [53]. In a typical experiment, 20 ml of chitosan solution 10 000 ppm as a stabilizer was first added to a 1000 ml beaker containing 180 ml of distilled water under stirring condition at a rate of 300 rpm. After the solution becomes homogenous; about

10 min of continuous stirring, 250 ml of $AgNO_3$ solution 1000 ppm was added and the mixture was stirred at the same rate for 1 h. The experiment was then continued by the increase in stirring rate to 500 rpm and the addition of 200 ppm $NaBH_4$ solution (20 ml) as a reductant by dropwise method. As soon as the $NaBH_4$ solution was dropped, the solution in the beaker turned yellow and became darkened as more reductants were added. The experiment finished as the addition of $NaBH_4$ completed. The SNP solution obtained was transferred to brown sample bottle and kept at room condition for later investigations.

2.3. Disinfection study

Disinfection ability of SNPs was investigated in the lab using *Salmonella* and in pig house using the index of microbial air contamination as indicators, respectively. For the antibacterial test in the lab, *Salmonella* was first grown by adding 100 μl of stock into BHI medium and incubated for 6 h at 37 °C following by centrifugation to separate supernatant. The bacterial cell pellet was then dispersed in sterilized distilled water to obtain a *Salmonella* suspension for later use. The *Salmonella* suspension (0.5 ml) was subsequently mixed with 0.5 ml SNPs solution having different concentrations from 0.025 to 250 ppm in a 1.5 ml tube. After incubation at 37 °C for 1 h, the suspension (100 μl) was taken and diluted with sterilized distilled water by serial ten-fold dilutions and then 100 μl of each dilution was extracted and streaked on NA plate, incubated for 24 h at 37 °C for bacterial colony count. The control experiment was conducted using the same method but the sterilized distilled water was added instead of SNPs solution.

The number of bacteria in the sample was calculated using equation (1).

$$N = \frac{C}{V(n_1 + 0.1n_2)d} \tag{1}$$

where, N is the total number of bacteria in a mL (CFU/mL), C is total colony counted on the plates of two consecutive dilutions, V is the volume of sample streaked on each plate, n_1, n_2 are the number of plates for the first and second dilution, respectively, and d is the dilution factor of the first dilution.

The index of microbial air contamination in pig house was investigated using a passive method called settle plate method. Accordingly, PCA medium containing petri plates (9 cm in diameter) were placed in four corners and center of pig house at the height of 40 cm from floor and 1 m away from walls or obstacle and exposed to air for 10 min The experiment began with the site preparation in which the floor was cleaned by water and left until it dried for the settle plate test. When the floor got dried, the control settle plate test was conducted by opening the petri plates for 10 min, afterward; the SNP solution with concentration of 25 ppm was sprayed throughout the floor surface and on the wall. As the floor got dried against, another settle plate test was conducted by opening the plates for 10 min After collecting, the plates were sealed to prevent the post contamination and incubated for 24 h at 37 °C for bacterial colony count. The total aerobic microbes in a cubic meter of air were calculated according to equation (2).

$$X = \frac{A \cdot 100 \cdot 100}{S \cdot K} \tag{2}$$

where, X is total number of bacteria in a cubic meter, A is an average number of bacteria counted, S is the area of the plate, K is a factor related to exposure time; it is 1, 2, and 3 corresponding to 5, 10, and 15 min, respectively, and 100 is a correlation factor to convert cm^2 to m^3.

2.4. Cell culture and virus preparation

Primary porcine alveolar macrophages (PAMs) were collected from lung of 6–8 week, 20–40 kg, and healthy Large White pigs. The alveolar cells were cultured in RPMI media with 10% swine serum and 1% antibiotic. For mono-layer cultures, alveolar cells were seeded in tissue culture plastic plates at about $4{\cdot}10^5$ cell cm^{-2}. After 24 h at 37 °C in moist air with 5% CO_2, non-adherent cells were removed by washing with medium.

VNUA/HY-ASF1, a p72 genotype II virus originated from infected pigs in a farm in Hung Yen province, Vietnam, which was extracted and cultured to a titer of 10^6 HAD_{50} as reported in a previous work, was used for this investigation [51].

2.5. Cell toxicity test

The SNP stock (500 ppm) was diluted to various concentrations ranging from 0.024 ppm to 50 ppm to perform the cell toxicity test. The PAM cell previously seeded in a 96-well plate was decanted to eliminate the medium following by the addition of 50 μl diluted SNP solution and 50 μl RPMI medium to each well. Final SNP concentration in the cell homogenate is calculated by dividing diluted SNP concentration added to the well by 2 due to the (1:1) dilution. Afterward, the plate was incubated for about 1 h at 37 °C in 5% CO_2 atmosphere and then 200 μl fresh medium was supplemented to monitor the cell viability. The dead and alive cells were determined by the identification of their morphological variation following the treatment with SNPs. The live

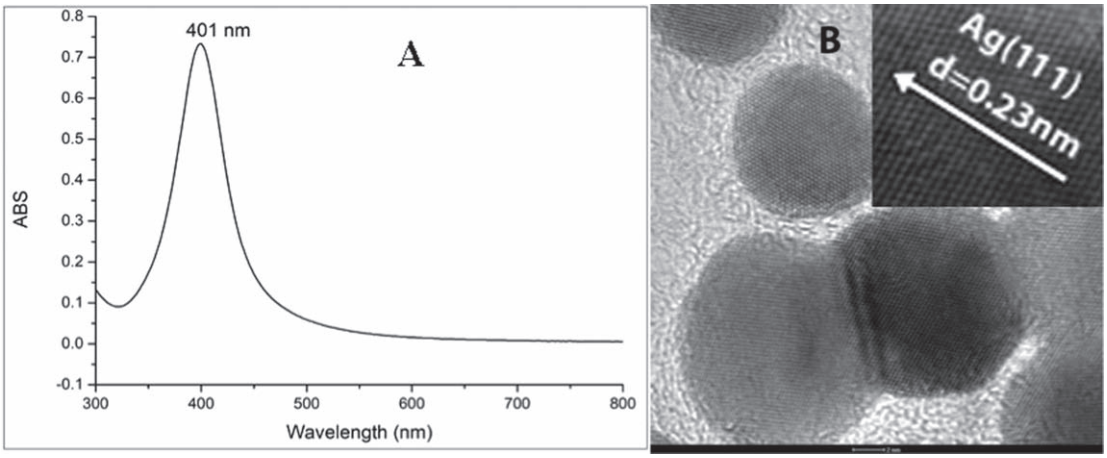

Figure 1. UV-Vis spectrum corresponding to the surface Plasmon resonance (A) and TEM image of the synthesized SNPs (B).

ones appear in a clear and spherical shape as the ones in the control, while the dead ones are shrunk and sometimes are in a non-spherical shape or broken up.

2.6. Viral inhibition test

In order to examine the inhibitory activity of SNPs on the virus, the highest non-toxic concentration of SNP solution was selected and mixed with virus supernatant at a volumetric ratio of 1:1. In a typical experiment, a virus with a titer of 10^6 HAD_{50} was diluted by medium using serial ten-fold dilutions prior to mix with SNP solution. The virus supernatant after being mixed with SNPs was kept at room temperature for 1 h for further experiments. Experiments then were followed by the addition of 100 μl solution having both SNPs and virus at different titers into wells containing cell after eliminating medium. The controls were prepared by growing cells in medium only and by the addition of virus only. The plate was incubated at 37 °C in 5% CO_2 for about 8 h for the survived virus to replicate. Following the incubation, the mixture of virus and SNPs was separated from the cells and washing with PBS 1X solution and then 200 μl medium with the complement of porcine red blood cell 1% was added. Finally, the cells were incubated at 37 °C for 7 days and observed on a microscope to evaluate their growth based on their shape alteration and the characteristic rosette formation representing haemadsorption of erythrocytes around infected cells. The antiviral activity of SNPs was determined based on the viability of cells at the studied titer in the absence of cytopathic effect (CPE).

2.7. Characterization

Transmission electron microscopy (TEM) was performed using FEI Tecnai G2 at an acceleration voltage of 200 kV. Samples were first dispersed in ethanol, deposited on carbon film supported by Cu grid, and then mounted on TEM for analysis. The collected TEM images were used to analyze the morphology, particle sizes and the nanostructure of SNPs. The morphology of cells was observed on a Leica DM IL LED microscope (Leica Microsystem). UV-Vis spectra were collected on a Hitachi 5300 H UV-Vis spectrophotometer to observe the formation of SNPs during synthetic process.

3. Results and discussion

The $AgNO_3$ solution turned yellow right after the $NaBH_4$ solution was added indicating the possible formation of SNPs. This phenomenon could relate to the characteristic surface Plasmon resonance of SNPs. The characterization on the UV-Vis spectraphotometer revealed that the absorption reached a maximum at the incident wavelength of 401 nm (figure 1(A)), which coincided with the surface Plasmon resonance of SNPs reported in many previous works [25, 54, 55]. The darkened solution observed when the more $NaBH_4$ solution added is due to the increase in SNP concentration, which is proportional to the absorption intensity. This was demonstrated in previous studies [56, 57] in which the evolution of SNPs was monitored by using UV-Vis spectra collected at different reaction time. The position of absorption peak may slight vary in different studies due to the nanoparticle size and shape effects; however, this is a facile method for the initial and rapid evaluation of SNP formation. To further confirm the presence of SNPs, high resolution TEM images (figure 1(B)) were taken for the analyses of their size, shape, and crystallinity. TEM image analysis revealed that the obtained particles have spherical shape with an approximate average diameter of 14 nm. These particles are highly

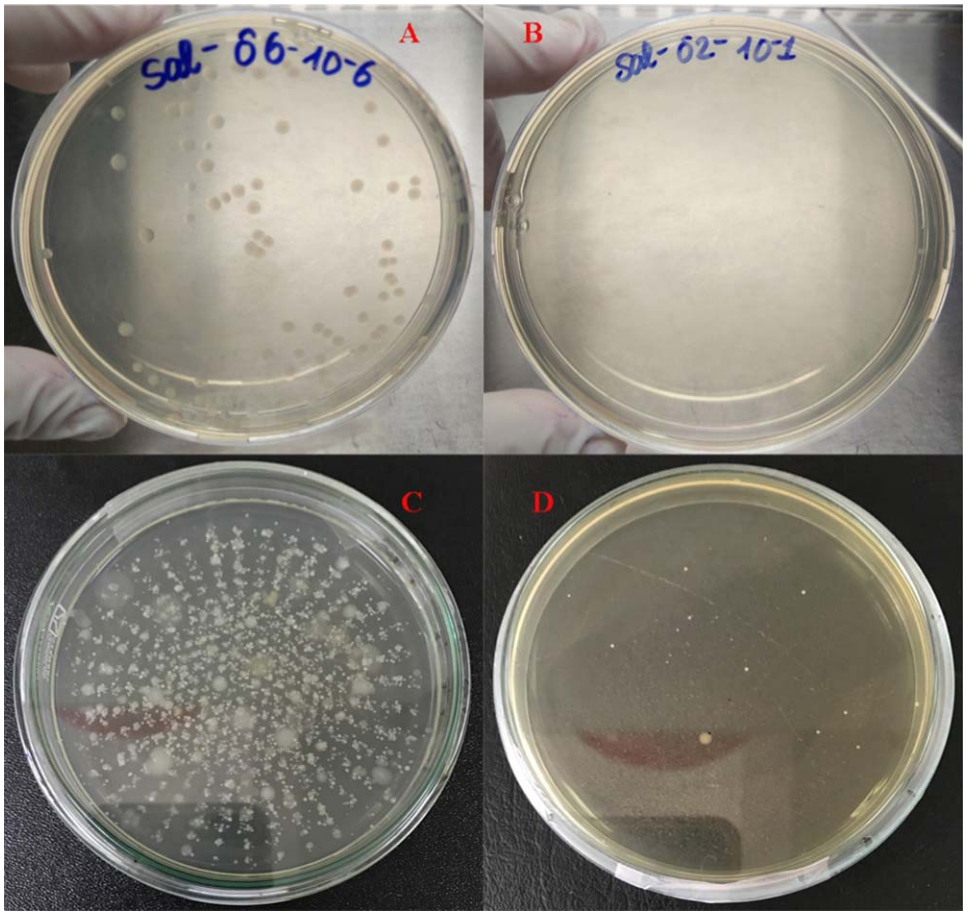

Figure 2. Antibacterial ability of SNPs against *Salmonella*; colonies from a control sample diluted at 10^6 times (A) and sample treated with SNPs 25 ppm diluted at 10 times (B), and microbial contamination tested by a settle plate method; bacterial colonies grew on agar plate exposed to the air for 10 min before (C) and after (D) spraying with SNPs 25 ppm.

crystalline with a d spacing of 0.23 nm (figure 1(B) inset), corresponding to (111) plane of metallic silver. This result demonstrated that metallic SNPs have been successfully synthesized and can be used for later experiments.

The antibacterial ability of synthesized SNPs was examined against *Salmonella* at different concentrations from 0.025 to 250 ppm and results are shown in figures 2 and 3. The control sample without adding of SNPs contains 1.23×10^8 CFU ml^{-1}, meanwhile no bacterium was found in the sample treated with SNPs at the concentration above 25 ppm (figure 2). As seen in figure 3, SNPs can inhibit more than 95% *salmonella* at the SNP concentration of 2.5 ppm and reach 100% at the SNP concentration of 25 ppm. The antibacterial efficiency appeared to be concentration-dependent, which reduced from 100% to 44.7% as SNP concentration decreased from 25 to 0.025 ppm, respectively. The mechanism that accounts for antibacterial property of SNPs are not fully elucidated, but there is a wide consensus that SNPs can inhibit bacteria through three mechanisms: (1) Ag^+ ions disrupt ATP production and DNA replication, (2) SNPs and Ag^+ ions generate excess radical oxygen species (ROS) that breakdown membrane and mitochondrial function or cause DNA damage, and (3) SNPs interact and break bacterial cell membrane [18]. The results revealed that SNPs is effective antibacterial agent and could be potential disinfectant for anti-ASFV.

3.1. Cell toxicity

To evaluate the toxicity of SNPs on PAM cell, SNPs at various concentrations were added into the wells containing cells to observe their growth. The layout of cell toxicity assay in a 96-well plate is depicted in figure 4. Each column has 7 wells for each SNP concentration (row A to G) and the 8^{th} well (row H) was a blank without SNPs for control. The toxic effect was observed at the SNP concentration of 1.56 ppm with approximately 70% cell deactivated. Above 1.56 ppm, SNPs show a high toxicity with 100% cells deactivated, while at more diluted concentration ($\leqslant$0.78 ppm), the toxic effect was not observed with over 80% cell growing normally. The observation on microscope (figure 5) showed that cells in the control sample have clear spherical shape, which is similar to samples treated with SNP concentration of 0.78 ppm and below, meanwhile cells in samples treated

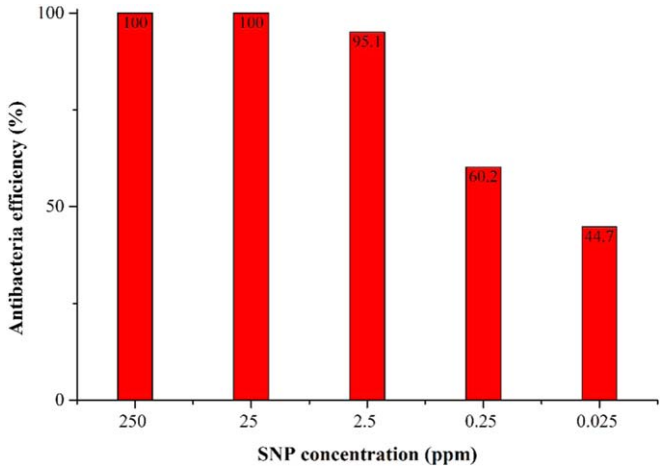

Figure 3. Antibacterial efficiency of SNPs against Salmonella.

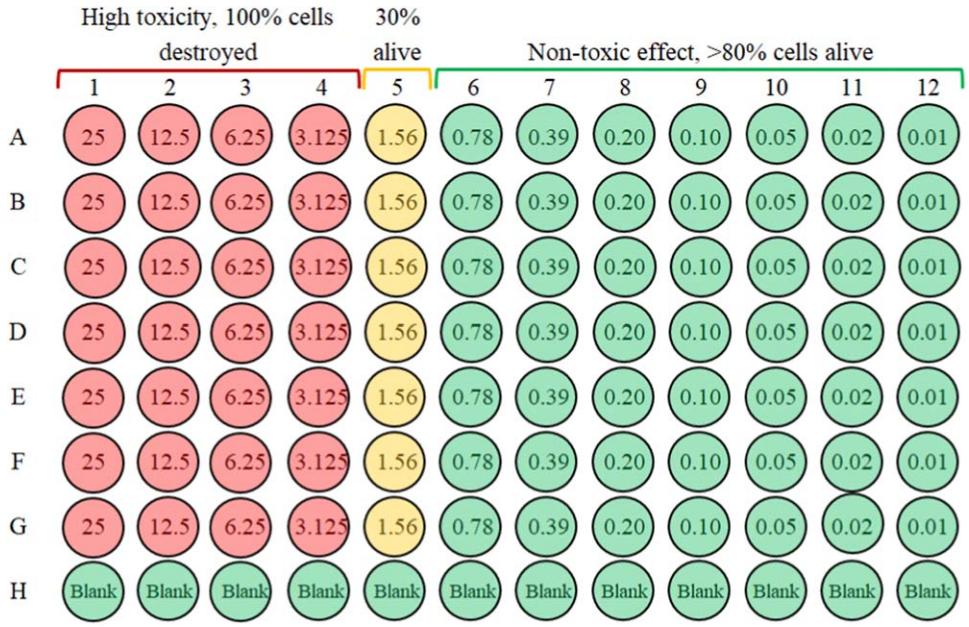

Figure 4. A schematic layout of the cell toxicity assay. Each column has 7 wells from row A to G for each SNP concentration and the 8th well in row H was for a control without SNPs. Red indicates high toxicity, which causes cell deactivation, yellow shows toxicity with high ratio of cell deactivated, and green reveals safe SNP concentration with more than 80% of cells alive.

with SNPs 1.56 ppm and above were clearly broken up. This asserted that SNPs at the concentration of 0.78 ppm and below are non-toxic to PAM cells.

The toxicity caused by Ag^{+} has been investigated over past 50 years and its mechanism has been well known with a general consensus that mitochondria are primary target of Ag^{+}. Mitochondria are vulnerable to the 'permeability transition pathway', which characterized by the formation of proteinaceous pores in mitochondrial membranes. The lowest level for Ag^{+} that may induce adverse effect in mammalian cells has been observed are from 222 to 362 mg Ag $K^{-1}g^{-1}$-day [58]. The toxicity caused by SNPs is not similar to that by Ag^{+}; there are number of factors that enable SNPs to deliver toxic effects to cells and organism. SNPs can penetrate cell walls and membranes and then release intracellular Ag^{+}. The Ag^{+} here can directly interact with DNA causing cytotoxic and genotoxic effects due to the disruption of cell transport and depletion of glutathione and other anti-oxidants [59, 60]. The SNPs can stimulate the production of ROS and decrease the ATP production causing oxidative stress and genotoxic effects. Since most of toxic effects occur intracellular, it is believed that the toxicity of SNPs is size-dependent; which could be more toxic in smaller size particles, particularly particles with size $\leqslant$5 nm [61].

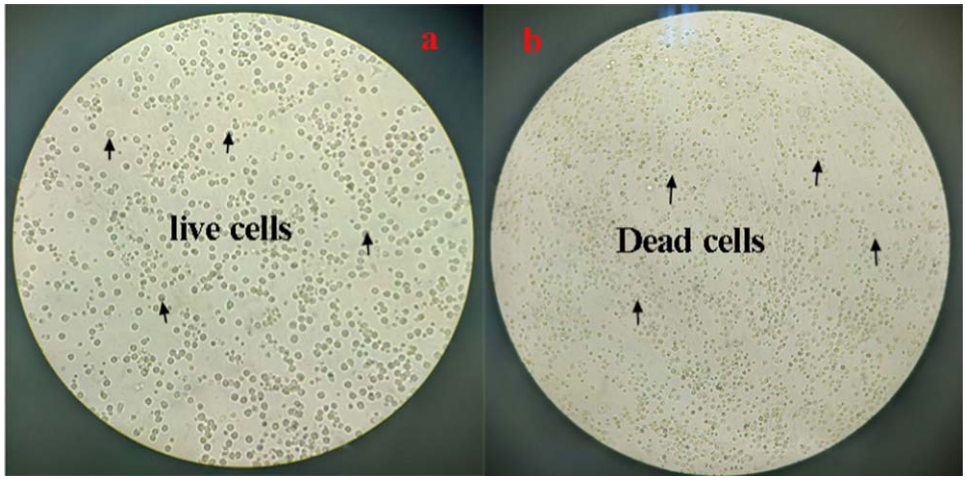

Figure 5. Representative images of the control sample with normal cells (a) and dead cells in sample treated with SNPs at 3.125 ppm (b) Images were taken on a Leica DM IL LED microscope at a magnification of 200×.

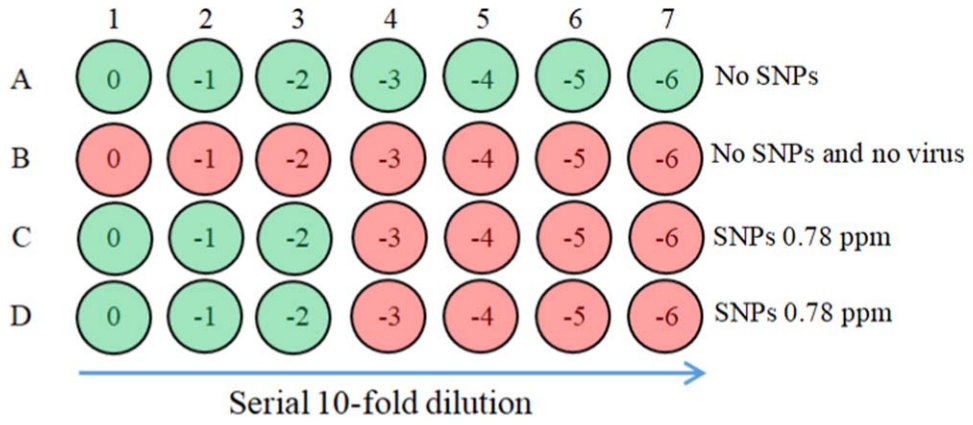

Figure 6. A schematic layout of antiviral experiment. Row A, B, C, D correspond to a blank sample with virus and no SNPs treatment (A), a control without SNPs and without virus (B), and viral samples treated with SNPs 0.78 ppm (C and D), respectively. Columns from 1 to 7 correspond to serial 10-fold dilution of virus homogenate at a titer of 10^6 HAD_{50}; minus numbers in circles represent to the log of dilution. Green circles indicate live virus detected, while red circles indicate no live virus detected.

The toxicity of SNPs is concentration-dependent and its effect may vary on the type of cells [59]. The viability of RAW 246.7 cell reduced 20% and 40% at SNPs concentration of 0.2 and 1.6 ppm, respectively [62]. The toxicity also observed on rat liver cell line (BRL 3 A) at the SNPs concentration range of 1 to 25 ppm [63]. The toxicity threshold of SNPs measured on HeLa and U937 cells is 2 ppm after 4 h treatment [64]. However, no toxicity was found on HepG2 cell at SNPs concentration from 0.01 to 5ppm [65]. Microscopic observation on porcine skin treated with SNPs for 14 days exhibited that their toxicity on porcine skin is a concentration-dependent response [66]. A slight intracellular and intercellular epidermal edema was found on the skin treated with 0.34 ppm SNPs (20 nm in size) and a severe focal dermal inflammation was observed as SNP concentration increased to 34 ppm. Obviously, the toxicity of SNPs varies significantly with different cell lines, therefore, depending on their application purposes; the toxicity threshold on involved cell lines should be investigated. In this study, the observed toxicity threshold (0.78 ppm) allows us to further investigate the antiviral activity of SNPs on the ASF virus while avoiding the damage of PAM cells.

3.2. Antivirus activity

Antiviral activity of SNPs on ASFV was investigated at the SNPs concentration of 0.78 ppm, the toxicity threshold of SNPs. A schematic layout of antiviral experiment is exhibited in figure 6. Viruses were detected in all dilutions when the initial virus homogenate was not treated with SNPs (green circles), whereas, no virus was found in the control without virus and without SNPs (Red circles). The differences can be seen in the sample treated with SNPs 0.78 ppm. No virus was detected when the stock homogenate was diluted by 3 log and above (Red circles), while it was observed in all dilutions below 2 log. These results indicated that ASFV at a titer of

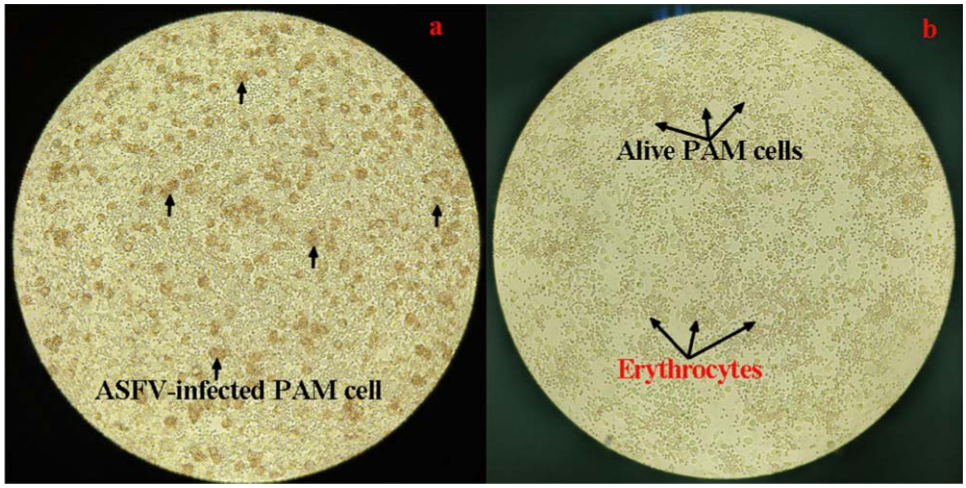

Figure 7. Representative microscopic images of PAM cells inoculated with ASFV (a) and PAM cells inoculated with ASFV treated in 0.78 ppm SNP solution for 1 h (b) Images were taken on Leica DM IL LED microscope at a magnification of 200×.

$\leqslant 10^3$ HAD_{50} can be completely inhibited by the SNPs at the concentration of 0.78 ppm. Previous studies revealed that the antiviral efficiency of SNPs is concentration-dependent [29, 33, 36, 43, 44], thus, their antiviral efficiency can be higher as SNP concentration increased; however, it could be more cytotoxic at higher concentrations. Images of infected-cells and normal PAM cells are shown in figure 7. Obviously, without treatment with SNPs, its virulent causes infection and death in PAM cells. As can be seen in figure 7(a), the cells in the sample, where virus was not treated with SNPs 0.78 ppm, have morphology altered and show the erythrocyte adsorption around infected cells. The erythrocyte adsorption is due to the interaction between erythrocytes and viral glycoprotein [67]. Meanwhile, PAM cells were found live with round clear shapes and no erythrocyte adsorption in blank and in virus homogenate diluted by $\geqslant$3 log following by the treatment with 0.78 ppm SNPs (figure 7(b)). The result further confirms the inhibitory ability of SNPs against ASFV.

According to previous studies, SNPs can interact with viral capsid gp120 glycoprotein, particularly the glycoprotein knobs, the more exposed and accessible parts on viral capsid and thereby prevent the penetration between virus and host cell and consequently induce an efficient inhibitory activity against viruses [29–31]. This mechanism can explain for viral inhibitory effect on a number of viruses having glycoprotein on particle membrane including H1N1 influenza A [29–31], H3N2 influenza viruses [32], and parainfluenza virus type 3 [38]. SNPs can also inhibit the formation of intracellular RNA and extracellular virions by interaction with HBV double-stranded DNA or viral particles [36]. ASFV is an enveloped DNA virus with particles ranging from 170 to 190 nm in diameter packed with more than 50 proteins including glycoprotein [67]. Therefore, it is likely that SNPs can interact with these proteins, particularly glycoprotein on the exterior membrane, preventing the virus entry into cells or the virus replication and thereby cause the viral inhibition.

Since the viral inhibition is based on the interaction between SNPs and viral protein, the antiviral efficiency depends significantly on the exposure of active sites on both viral particle and SNP. Accordingly, the size of SNPs and the capping agent used during the synthesis may have important contribution on the antiviral efficacy of the resulting SNPs. Gaikwad *et al* demonstrated that SNPs with mean diameter of 47 nm and 45 nm produced by fungies, Alternaria species and Phoma species, respectively, have low viral inhibition (between 0 and 40%), meanwhile SNPs produced by F. oxysporum (24 nm) and C. Indicum (45 nm) have inhibitory efficiency up to 80% (F. Oxysporium) and 90% (C. Indicum) [38]. A higher antiviral activity against RSV can also be received when SNPs stabilized by curcumin in compared with citric acid [34]. In this study, SNPs having an approximate average diameter of 14 nm can completely inhibit ASFV at a titer of 10^3 HAD_{50} *in vitro*. However, the antiviral efficiency in reality can be greatly affected by contaminants existing in the environment. The adverse effect could be more serious when SNPs are applied for pig house facility cleaning and disinfection since there are a lot of organic compounds from leftover food and pig excretion that could strongly interact with SNPs. To evaluate this effect on the disinfection ability of SNPs in the pig house, SNPs solution 25 ppm has been sprayed on the floor, wall and partition of pig barn and then bacterial contamination in air before and after spraying was monitored by a passive method called settle plates [68]. Tests were conducted in 3 different barns for piglets, sows, and adult pigs, respectively. Before spraying SNPs solution, bacteria growth on plates are so much that could not be counted as can be seen in figures 2(C) and (D), however, after spraying with SNPs solution, the average number of bacteria on plates are 34, 95, and 813 cfu/plate corresponding to 2674, 5760, and 63914 cfu m^{-3} of air in barns for sows, piglets, and adult pigs, respectively. The results revealed that microbial contamination reduced

significantly after spraying with SNP solution 25 ppm. The higher numbers of bacteria received in piglets and adult pig barns do not mean that the disinfection efficiency of SNPs reduced in those cases. It is likely that the bacteria concentration in the air vary with the active level of pigs in the house; the pig activity creates and pumps more aerosols with bacteria into the atmosphere. In sow barn, each one is separated in a single partition with very limited activity; it spends more time lying on floor and therefore causes fewer aerosols with bacteria flushing in the air. As a result, fewer bacteria are deposited on agar plates. Whereas, piglets and adult pigs are freely moved in barn space causing more aerosols with bacteria supplying into the air and as consequence, more bacteria were found deposited on agar plates. The obtained results confirmed that the disinfection activity of SNPs is not significant influenced by contaminants remaining on the floor.

SNPs have been widely used as an antibacterial agent in many applications from textile industry, water disinfection, food packaging to medicine [19]. SNPs have some advantages compared to conventional disinfectants. They have strong and long-lasting antibacterial activity against wide range of microbes including bacteria, fungi, and viruses. They can be easily incorporated with other substrates and materials; therefore, they can be used in the form of liquid or solid. Thus, SNPs offer numerous applications water disinfection, air filtration, surface disinfection, lotion or ointment for skin, pad or cloth for wound care. Thanks to a versatile application and a long-lasting activity, SNPs can create an effective barrier to break the transmission of ASFV. This suggests that SNPs could be a potential disinfectant and an effective tool to prevent the rapid widespread of ASFV.

4. Conclusion

This study demonstrated that SNPs are an effective disinfectant against both *Salmonella* and ASFV. The complete inhibition of the *Salmonella* bacteria and ASFV was observed at the SNP concentration of 25 and 0.78 ppm and at the bacterial concentration of 10^8 CFU ml^{-1} and viral titer of 10^3 HAD_{50}, respectively. SNPs do not show cytotoxicity to the PAM cells at the concentration of 0.78 ppm. The results confirmed that SNPs have a strong antiviral ability against ASFV and can be a promising tool to fight against the disease widespread.

To control the widespread of ASFV, besides a demand for an effective vaccine, it requires the implementation of many biosecurity measures to isolate the outbreak areas, to disinfect the infected areas, and to create protective boundaries for uninfected areas. SNPs could have a great contribution in the implementation of the biosecurity measures; however, the use of SNPs should be combined with existing preventive measures to optimize the cost and their effectiveness. Therefore, further studies, particularly the field studies should be carried out to achieve an effective combination of SNPs with existing preventive measures and to reduce the cost of preventive measures.

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Data availability

The raw/processed data required to reproduce these findings cannot be shared at this time due to technical or time limitations.

ORCID iDs

Dang Viet Quang https://orcid.org/0000-0003-2816-0316

References

[1] Galindo I, Hernáez B, Berná J, Fenoll J, Cenis J L, Escribano J M and Alonso C 2011 Comparative inhibitory activity of the stilbenes resveratrol and oxyresveratrol on African swine fever virus replication *Antiviral Research* **91** 57–63
[2] Adriana V D, Christopher L N, Linda K D and Anthony J W 2012 African swine fever virus strain georgia 2007/1 in *Ornithodoros erraticus* ticks *Emerging Infectious Disease journal* **18** 1026
[3] Brown V R and Bevins S N 2018 A review of African swine fever and the potential for introduction into the United States and the possibility of subsequent establishment in feral swine and native ticks *Frontiers in Veterinary Science* **5** 11–11
[4] Golnar A J *et al* 2019 Reviewing the potential vectors and hosts of african swine fever virus transmission in the United States *Vector-Borne and Zoonotic Diseases* **19** 512–24

[5] Sánchez-Cordón P J, Montoya M, Reis A L and Dixon L K 2018 African swine fever: a re-emerging viral disease threatening the global pig industry *The Veterinary Journal* **233** 41–8
[6] World Organization for Animal Health (OIE) 2019 African Swine Fever, Technical Disease Cards https://www.oie.int/fileadmin/Home/eng/Animal_Health_in_the_World/docs/pdf/Disease_cards/AFRICAN_SWINE_FEVER.pdf
[7] Jurado C, Martínez-Avilés M, De La Torre A, Štukelj M, de Carvalho Ferreira H C, Cerioli M, Sánchez-Vizcaíno J M and Bellini S 2018 Relevant measures to prevent the spread of African swine fever in the european union domestic pig sector *Frontiers in Veterinary Science* **5** 1–16
[8] Krug P W, Lee L J, Eslami A C, Larson C R and Rodriguez L 2011 Chemical disinfection of high-consequence transboundary animal disease viruses on nonporous surfaces *Biologicals* **39** 231–5
[9] Juszkiewicz M, Walczak M and Woźniakowski G 2019 Characteristics of selected active substances used in disinfectants and their virucidal activity against ASFV *J Vet Res* **63** 17–25
[10] Zakaryan H and Revilla Y 2016 African swine fever virus: current state and future perspectives in vaccine and antiviral research *Veterinary Microbiology* **185** 15–9
[11] Galindo I and Alonso C 2017 African swine fever virus: a review *Viruses* **9** 103
[12] Arabyan E *et al* 2018 Genistein inhibits African swine fever virus replication *in vitro* by disrupting viral DNA synthesis *Antiviral Research* **156** 128–37
[13] García-Villalón D and Gil-Fernández C 1991 Antiviral activity of sulfated polysaccharides against African swine fever virus *Antiviral Research* **15** 139–48
[14] Hakobyan A, Arabyan E, Avetisyan A, Abroyan L, Hakobyan L and Zakaryan H 2016 Apigenin inhibits African swine fever virus infection *in vitro Arch. Virol* **161** 3445–53
[15] Mottola C, Freitas F B, Simões M, Martins C, Leitão A and Ferreira F 2013 *In vitro* antiviral activity of fluoroquinolones against African swine fever virus *Veterinary Microbiology* **165** 86–94
[16] Frouco G, Freitas F B, Martins C and Ferreira F 2017 Sodium phenylbutyrate abrogates African swine fever virus replication by disrupting the virus-induced hypoacetylation status of histone H3K9/K14 *Virus Research* **242** 24–9
[17] Franci G, Falanga A, Galdiero S, Palomba L, Rai M, Morelli G and Galdiero M 2015 Silver nanoparticles as potential antibacterial agents *Molecules* **20** 8856–74
[18] Marambio-Jones C and Hoek E M V 2010 A review of the antibacterial effects of silver nanomaterials and potential implications for human health and the environment *J. Nanopart. Res.* **12** 1531–51
[19] Tran Q H, Nguyen V Q and Le A-T 2013 Silver nanoparticles: synthesis, properties, toxicology, applications and perspectives *Advances in Natural Sciences: Nanoscience and Nanotechnology* **4** 033001
[20] Ngoc Dung T T, Phan Thi L-A, Nam V N, Nhan T T and Quang D V 2019 Preparation of silver nanoparticle-containing ceramic filter by *in-situ* reduction and application for water disinfection *Journal of Environmental Chemical Engineering* **7** 103176
[21] Pratheesya T, Harish S, N M, Sohila S and Ramesh R 2019 Enhanced antibacterial and photocatalytic activities of silver nanoparticles anchored reduced graphene oxide nanostructure *Materials Research Express* **6** 074003
[22] Mamo T, Moseman E A, Kolishetti N, Salvador-Morales C, Shi J, Kuritzkes D R, Langer R, von Andrian U and Farokhzad O C 2010 Emerging nanotechnology approaches for HIV/AIDS treatment and prevention *Nanomedicine (Lond)* **5** 269–85
[23] Lara H H, Garza-Treviño E N, Ixtepan-Turrent L and Singh D K 2011 Silver nanoparticles are broad-spectrum bactericidal and virucidal compounds *Journal of Nanobiotechnology* **9** 30
[24] Galdiero S, Falanga A, Vitiello M, Cantisani M, Marra V and Galdiero M 2011 Silver nanoparticles as potential antiviral agents *Molecules* **16** 8894–918
[25] Elechiguerra J L, Burt J L, Morones J R, Camacho-Bragado A, Gao X, Lara H H and Yacaman M J 2005 Interaction of silver nanoparticles with HIV-1 *Journal of Nanobiotechnology* **3** 6
[26] Lara H H, Ayala-Nuñez N V, Ixtepan-Turrent L and Rodriguez-Padilla C 2010 Mode of antiviral action of silver nanoparticles against HIV-1 *Journal of Nanobiotechnology* **8** 1–1
[27] Singh D K and Lara H H 2012 Inhibition of cell-associated HIV-1 by silver nanoparticles *Retrovirology* **9** O1-O1
[28] Baram-Pinto D, Shukla S, Perkas N, Gedanken A and Sarid R 2009 Inhibition of herpes simplex virus type 1 infection by silver nanoparticles capped with mercaptoethane sulfonate *Bioconjugate Chem.* **20** 1497–502
[29] Xiang D-x, Chen Q, Pang L and Zheng C-l 2011 Inhibitory effects of silver nanoparticles on H1N1 influenza a virus *in vitro Journal of Virological Methods* **178** 137–42
[30] Mori Y, Ono T, Miyahira Y, Nguyen V Q, Matsui T and Ishihara M 2013 Antiviral activity of silver nanoparticle/chitosan composites against H1N1 influenza a virus *Nanoscale Res. Lett.* **8** 93
[31] Li Y, Lin Z, Zhao M, Xu T, Wang C, Hua L, Wang H, Xia H and Zhu B 2016 Silver nanoparticle based codelivery of oseltamivir to inhibit the activity of the h1n1 influenza virus through ROS-mediated signaling pathways *ACS Applied Materials & Interfaces* **8** 24385–93
[32] Xiang D *et al* 2013 Inhibition of A/Human/Hubei/3/2005 (H3N2) influenza virus infection by silver nanoparticles *in vitro* and *in vivo Int J Nanomedicine* **8** 4103–13
[33] Sun R W-Y, Chen R, Chung N P Y, Ho C-M, Lin C-L S and Che C-M 2005 Silver nanoparticles fabricated in Hepes buffer exhibit cytoprotective activities toward HIV-1 infected cells *Chem. Commun.* 5059–61
[34] Yang X X, Li C M and Huang C Z 2016 Curcumin modified silver nanoparticles for highly efficient inhibition of respiratory syncytial virus infection *Nanoscale* **8** 3040–8
[35] Orlowski P *et al* 2014 Tannic acid modified silver nanoparticles show antiviral activity in herpes simplex virus type 2 infection *PLoS One* **9** e104113
[36] Lu L, Sun R, Chen R, Hui C, Ho C, Luk J, Lau G and Che C 2008 Silver nanoparticles inhibit hepatitis B virus replication *Antiviral therapy* **13** 253–62
[37] Sun L, Singh A K, Vig K, Pillai S R and Singh S R 2008 Silver nanoparticles inhibit replication of respiratory syncytial virus *Journal of Biomedical Nanotechnology* **4** 149–58
[38] Gaikwad S, Ingle A, Gade A, Rai M, Falanga A, Incoronato N, Russo L, Galdiero S and Galdiero M 2013 Antiviral activity of mycosynthesized silver nanoparticles against herpes simplex virus and human parainfluenza virus type 3 *Int J Nanomedicine* **8** 4303–14
[39] Huy T Q, Hien Thanh N T, Thuy N T, Chung P V, Hung P N, Le A-T and Hanh N T H 2017 Cytotoxicity and antiviral activity of electrochemical—synthesized silver nanoparticles against poliovirus *Journal of Virological Methods* **241** 52–7
[40] Chen N, Zheng Y, Yin J, Li X and Zheng C 2013 Inhibitory effects of silver nanoparticles against adenovirus type 3 *in vitro Journal of Virological Methods* **193** 470–7
[41] Speshock J L, Murdock R C, Braydich-Stolle L K, Schrand A M and Hussain S M 2010 Interaction of silver nanoparticles with Tacaribe virus *Journal of Nanobiotechnology* **8** 19

[42] Pangestika R and Ernawati R 2017 Antiviral Activity Effect of Silver Nanoparticles (Agnps) Solution Against the Growth of Infectious Bursal Disease Virus on Embryonated Chicken Eggs with Elisa Test *The Veterinary Medicine International Conference 2017* **2017** 536–48
[43] Lv X, Wang P, Bai R, Cong Y, Suo S, Ren X and Chen C 2014 Inhibitory effect of silver nanomaterials on transmissible virus-induced host cell infections *Biomaterials* **35** 4195–203
[44] Du T, Lu J, Liu L, Dong N, Fang L, Xiao S and Han H 2018 Antiviral activity of graphene oxide–silver nanocomposites by preventing viral entry and activation of the antiviral innate immune response *ACS Applied Bio Materials* **1** 1286–93
[45] Zhou X *et al* 2018 Emergence of African swine fever in China 2018 *Transboundary and Emerging Diseases* **65** 1482–4
[46] Hyun-Joo K, Min-Jung L, Soo-Kyoung L, Da-young K, Sang-Ji S, Hae-Eun K and Hyang-Mi N 2019 African Swine Fever Virus in Pork Brought into South Korea by Travelers from China August 2018 *Emerging Infectious Disease journal* **25** 1231
[47] Daesub S *et al* 2019 Outbreak of african swine fever, Vietnam, 2019 *Emerging Infectious Disease journal* **25** 1433–435
[48] Chenais E, Depner K, Guberti V, Dietze K, Viltrop A and Ståhl K 2019 Epidemiological considerations on African swine fever in Europe 2014-2018 *Porcine Health Manag* **5** 6–6
[49] Shao Y, Li M, Zhang W, Ji Y and Hayes D J 2018 World's largest pork producer in crisis: China's African swine fever outbreak *Agricultural Policy Review* **2018** 1–4
[50] Wang T, Sun Y and Qiu H-J 2018 African swine fever: an unprecedented disaster and challenge to China *Infect Dis Poverty* **7** 111–111
[51] Van Phan L *et al* 2019 Outbreak of African swine fever, Vietnam, 2019 *Emerging Infectious Disease journal* **25** 1433
[52] Dixon L K, Sun H and Roberts H 2019 African swine fever *Antiviral Research* **165** 34–41
[53] Chau N H, Bang L, Buu N, Dung T, Ha H and Quang D 2008 Some results in manufacturing of nanosilver and investigation of its application for disinfection *Advances in Natural Sciences* **9** 241–8
[54] Quang D V, Sarawade P B, Hilonga A, Kim J-K, Chai Y G, Kim S H, Ryu J-Y and Kim H T 2011 Preparation of amino functionalized silica micro beads by dry method for supporting silver nanoparticles with antibacterial properties *Colloids and Surfaces A: Physicochemical and Engineering Aspects* **389** 118–26
[55] Mogensen K B and Kneipp K 2014 Size-dependent shifts of plasmon resonance in silver nanoparticle films using controlled dissolution: monitoring the onset of surface screening effects *The Journal of Physical Chemistry* C **118** 28075–83
[56] Trapiella-Alfonso L, Menéndez-Miranda M, Costa-Fernández J M, Pereiro R and Sanz-Medel A 2014 Nanostructural transformations of silver nanoclusters occurring during their synthesis and after interaction with UV-light *Mater. Res. Express* **1** 015039
[57] Abdel-Fattah W I, Sattar A, Sallam M, Atwa N A, Salama E, Maghraby A M and Ali G W 2014 Functionality, antibacterial efficiency and biocompatibility of nanosilver/chitosan/silk/phosphate scaffolds 1. Synthesis and optimization of nanosilver/chitosan matrices through gamma rays irradiation and their antibacterial activity *Materials Research Express* **1** 035024
[58] Stensberg M C, Wei Q, McLamore E S, Porterfield D M, Wei A and Sepúlveda M S 2011 Toxicological studies on silver nanoparticles: challenges and opportunities in assessment, monitoring and imaging *Nanomedicine (Lond)* **6** 879–98
[59] Akter M, Sikder M T, Rahman M M, Ullah A K M A, Hossain K F B, Banik S, Hosokawa T, Saito T and Kurasaki M 2017 A systematic review on silver nanoparticles-induced cytotoxicity: physicochemical properties and perspectives *J. Adv. Res.* **9** 1–16
[60] Hussain S M, Hess K L, Gearhart J M, Geiss K T and Schlager J J 2005 *In vitro* toxicity of nanoparticles in BRL 3A rat liver cells *Toxicol. in Vitro* **19** 975–83
[61] Cho Y-M, Mizuta Y, Akagi J-I, Toyoda T, Sone M and Ogawa K 2018 Size-dependent acute toxicity of silver nanoparticles in mice *J Toxicol Pathol* **31** 73–80
[62] Park E-J, Yi J, Kim Y, Choi K and Park K 2010 Silver nanoparticles induce cytotoxicity by a Trojan-horse type mechanism *Toxicol. in Vitro* **24** 872–8
[63] Piao M J, Kang K A, Lee I K, Kim H S, Kim S, Choi J Y, Choi J and Hyun J W 2011 Silver nanoparticles induce oxidative cell damage in human liver cells through inhibition of reduced glutathione and induction of mitochondria-involved apoptosis *Toxicol. Lett.* **201** 92–100
[64] Kaba S I and Egorova E M 2015 *In vitro* studies of the toxic effects of silver nanoparticles on HeLa and U937 cells *Nanotechnol Sci Appl* **8** 19–29
[65] Mishra A R, Zheng J, Tang X and Goering P L 2016 Silver nanoparticle-induced autophagic-lysosomal disruption and NLRP3-inflammasome activation in HepG2 cells is size-dependent *Toxicol. Sci.* **150** 473–87
[66] Samberg M E, Oldenburg S J and Monteiro-Riviere N A 2010 Evaluation of silver nanoparticle toxicity in skin *in vivo* and keratinocytes *in vitro Environ. Health Perspect.* **118** 407–13
[67] Jia N, Ou Y, Pejsak Z, Zhang Y and Zhang J 2017 Roles of African swine fever virus structural proteins in viral infection *J Vet Res* **61** 135–43
[68] Pasquarella C, Pitzurra O and Savino A 2000 The index of microbial air contamination *Journal of Hospital Infection* **46** 241–56