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Antimicrobial efficacy of platinum-doped silver nanoparticles

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Abstract

Silver nanoparticles (AgNPs) have been proposed to combat oral infection due to their efficient ionic silver (Ag⁺) release. However, concentrations required for antimicrobial efficacy may not be therapeutically viable. In this work, platinum-doped silver nanoparticles (Pt-AgNPs) were explored to evaluate their potential for enhanced Ag⁺ release, which could lead to enhanced antimicrobial efficacy against *S. aureus*, *P. aeruginosa*, and *E. coli*. AgNPs doped with 0.5, 1, and 2 mol% platinum (Pt_{0.5}-AgNPs, Pt₁-AgNPs, and Pt₂-AgNPs) were synthesized by a chemical reduction method. Transmission electron microscopy revealed mixed morphologies of spherical, oval, and ribbon-like nanostructures. Surface-enhanced Raman scattering revealed that the surface of Pt-AgNPs was covered with up to 93% Pt. The amount of Ag⁺ released increased 16.3-fold for Pt₂-AgNPs, compared to AgNPs. The initial lag phase in bacterial growth curve was prolonged for Pt-AgNPs. This is consistent with a Ag⁺ release profile that exhibited an initial burst followed by sustained release. Doping AgNPs with platinum significantly increased the antimicrobial efficacy against all species. Pt₂-AgNPs exhibited the lowest minimum inhibitory concentrations, followed by Pt₁-AgNPs, Pt_{0.5}-AgNPs, and AgNPs, respectively. Doping AgNPs with a small amount of platinum promoted the release of Ag⁺, based on the sacrificial anodic effect, and subsequently enhanced their antimicrobial efficacy.

Keywords

antimicrobial activity; ionic silver release; minimum inhibitory concentration; platinum doping; silver nanoparticles

1 | INTRODUCTION

Increasing microbial resistance to antibiotherapy has long been identified as a global health threat, and there is a pressing need for developing alternate therapeutic agents efficient against antibiotic-resistant species (Zheng, Setyawati, Leong, & Xie, 2018). The antimicrobial activity of metals such as silver is well established (Chernousova & Eppe,

2013; Klasen, 2000a; Klasen, 2000b). Furthermore, the advancement of nanotechnology permitted the successful production of a large variety of metallic nanoparticles that have drawn interest as potential antimicrobial agents. Among these, silver nanoparticles (AgNPs) have been extensively studied, and their bactericidal properties over spectra of both Gram-positive and Gram-negative bacteria have been demonstrated (Espinosa-Cristóbal et al., 2009; Soo-Hwan, Lee, Ryu, Choi, & Lee, 2011; Yan et al., 2018). As a result, silver nanoparticles are successfully used in a variety of products, including specialized textiles, cosmetics, medical, and dental devices (Burdus et al., 2018; Chaloupka, Malam, & Seifalian, 2010; Chernousova & Eppe, 2013).

Although the precise mechanisms that explain the antimicrobial activity of silver nanoparticles have long been a subject of debate, three possible mechanisms have been proposed, including the adhesion of AgNPs onto the surface of bacteria. Adhered AgNPs impair the membrane integrity and are able to penetrate through the cell wall, where they further interact with intracellular biomolecules, resulting in bacterial cell death (McShan, Ray, & Yu, 2014; Morones et al., 2005; Sondi & Salopek-Sondi, 2004; Yan et al., 2018). Concurrently, silver nanoparticles become oxidized in the presence of oxygen-saturated aqueous solutions, and this results in the generation of the reactive oxygen species (ROS) and ionic silver (Ag^+). Reactive oxygen species are capable of interfering with bacterial antioxidant defense systems; glutathione (GSH) reductase, causing oxidative stress and killing the cells as a result (Carlson et al., 2008; Sun et al., 2016; Yan et al., 2018). Meanwhile, ionic silver shows a high affinity for thiolate ligand-containing proteins or enzymes present in bacterial cell membranes, as well as intracellular biomolecules, and phosphorus-containing DNA. This in turn, induces a down-regulation or inactivation of the corresponding metabolisms and leads to bacterial cell lysis (Carlson et al., 2008; Nel, Xia, Mädler, & Li, 2006; Sun et al., 2016; Tamayo, Zapata, Vejar, et al., 2014; Yan et al., 2018). While lower concentrations of silver nanoparticles are innocuous to mammalian cells, concentrations greater than $25 \mu\text{g}.\text{ml}^{-1}$ have been shown to induce some degree of toxicity (Han, Gurunathan, Jeong, et al., 2014; Xue et al., 2016). Furthermore, in vivo bio-distribution and toxicity studies have demonstrated that AgNPs interfered with genes associated with reproduction processes, and could cause significant toxicity in several organs, including the brain (Dobrzy ska et al., 2014; Kim et al., 2008). There is, therefore, some concern regarding the use of AgNPs as human therapeutic agents at concentrations greater than $25 \mu\text{g}.\text{ml}^{-1}$.

One approach to enhancing the antimicrobial activity of AgNPs while maintaining biocompatible levels is to dope silver nanoparticles with small amounts of a second metal, electrochemically less active than silver, creating a bimetallic nanoparticle (Gu, Zhang, & Tao, 2012). When placed in an electrolytic environment, the silver component of the bimetallic nanoparticle may act as a sacrificial anode and preferentially dissolve, promoting the release of ionic silver (Breisch et al., 2019; Narozny, Zakowski, & Darowicki, 2014). The electrocatalytic reaction occurs more readily when both metals are present at the surface of the particles (Li & Yamauchi, 2013). Candidates of choice as dopants with a higher electrochemical potential than silver, combined with excellent biocompatibility, are gold and platinum.

Several studies have examined the combined effects of silver and platinum on antimicrobial efficacy (Breisch et al., 2019; Chang et al., 2019). Chang et al. demonstrated that alloying AgNPs with approximately 11 mol% of gold, platinum, or palladium promoted the release of Ag^+ . It was shown that alloyed AgNPs inhibited the bacterial growth of *S. aureus* and *E. coli* (Chang et al., 2019). In contrast, Breisch et al., studied the antimicrobial activity of bimetallic silver-platinum nanoparticles with compositions ranging from 10 to 90 mol % platinum (Breisch et al., 2019). The antimicrobial activity was assessed against *S. aureus* and *E. coli* and compared to that of undoped silver and platinum nanoparticles. Results showed that only unalloyed AgNPs, or AgNPs alloyed with up to 50 mol% platinum exhibited antimicrobial activity. Furthermore, alloying with platinum did not enhance the antimicrobial activity of silver nanoparticles. However, it should be noted that platinum concentrations lower than 10 mol% were not investigated in these studies (Breisch et al., 2019; Chang et al., 2019).

The purpose of the present study was to investigate the antimicrobial efficacy of silver nanoparticles doped with platinum amounts ranging from 0.5 to 2 mol%. The hypothesis tested was that based on the sacrificial anode principle, Pt-doped AgNPs would lead to a larger release of silver ions than undoped particles and promote enhanced antimicrobial efficacy.

2 | MATERIALS AND METHODS

All chemicals were of analytical grade and were used without further purification.

Nanoparticle synthesis: AgNPs were synthesized by a chemical reduction method (Shrestha & Haes, 2015). In brief, silver perchlorate solution (0.1 mM) was mixed with a freshly prepared solution of trisodium citrate dihydrate (0.3 mM) and sodium borohydride (1 mM) in DI water (4°C) under vigorous stirring for 3 minutes. Platinum nanoparticles (PtNPs) were synthesized according to a modified protocol (Bigall et al., 2008). Potassium tetrachloroplatinate solution (0.1 mM) was mixed with a freshly prepared solution of trisodium citrate dihydrate (0.3 mM) and sodium borohydride (1 mM) in DI water. The mixture was maintained at 90°C for 30 minutes. Silver nanoparticles doped with 0.5, 1, and 2 mol% platinum ($\text{Pt}_{0.5}\text{-AgNPs}$, $\text{Pt}_1\text{-AgNPs}$, and $\text{Pt}_2\text{-AgNPs}$) were synthesized from an aqueous solution of silver perchlorate (0.1 mM) and potassium tetrachloroplatinate (0.0005, 0.001, and 0.002 mM), followed by mixing with trisodium citrate dihydrate (0.3 mM) and sodium borohydride (1 mM) under vigorous stirring for 3 minutes. All nanoparticles solutions were washed thrice with DI water and centrifuged (3500G) to remove unreacted chemicals. Nanoparticle pellets were then resuspended in DI water and kept at 4°C until used.

Ionic silver solution preparation: Ionic silver in aqueous solution was prepared by an electrolytic method. The electrolyte was prepared by dissolving silver nitrate in DI water with a final concentration of 67 mg.ml^{-1} . Both electrodes were made of platinum. First, the electrolytic process was performed at 200 V, 80 mA for 10 minutes to deposit silver on the working electrode (EC250–90 Electrophoresis power supply, ThermoEC). After completing the electrolytic process, both electrodes were switched, and polarities were inverted. The

now silver-coated platinum electrode served as a counter electrode, and the non-coated platinum electrode served as a working electrode. The electrolysis process was repeated at 200 V, 80 mA for 4 minutes, to promote the release of ionic silver in solution.

2.1 | Nanoparticle characterization

Extinction spectra of nanoparticles were collected using 10 mm optical path length quartz cuvettes with an ultraviolet–visible (UV–vis) spectrometer (Cary 50, Varian). Nanoparticles dimension, morphology, and specific surface area were characterized by transmission electron microscopy (TEM-1230, JEOL) at an accelerating voltage of 120 keV. TEM samples were prepared by adding 5 μ l of nanoparticle solutions diluted in bovine serum albumin on formvar/carbon-coated 200 mesh copper grids (Michen et al., 2015). Mean aspect ratio and specific surface area were determined by simplified geometric calculation. Silver and platinum concentrations were determined by flame atomic absorption spectroscopy (AAS, Perkin Elmer 2,380) at an operating current of 2 mA and spectral bandwidth of 0.2 nm. Silver and platinum hollow-cathode-lamps were used with wavelengths of 328.1 nm and 265.9 nm, respectively. A series of standard ionic solutions were prepared to establish calibration curves and further determine platinum and silver concentrations. Surface-enhanced Raman spectroscopy (SERS) in combination with 2,6-dimethylphenyl isocyanide (2,6-DMPI), a molecular probe used to detect the nucleation of platinum dopant on the surface of AgNPs, by following the stretching frequencies of the isocyanide (NC) bonds for silver and platinum ($\nu_{\text{NC(Ag)}}$ and $\nu_{\text{NC(Pt)}}$) (Zhang et al., 2017). The premise of this technique is that the isocyanide group binds differently to silver and platinum, resulting in a stronger bond with silver (blue shift) and a weaker bond with platinum (red shift). As-prepared nanoparticles were mixed with 1 ml of ethanol containing 2,6-DMPI (10^{-4} M) and incubated at 25°C for 60 minutes. 2,6-DMPI-functionalized nanoparticles were washed thrice and resuspended in DI water. SERS spectra were recorded in the solution phase at a power of 7.98 mW with a 532 nm excitation laser and a collection time of 10 s per sample (ExamineR 532 spectrometer, DeltaNu). The amount of ionic silver released from AgNPs and Pt-AgNPs was measured with an ion-selective electrode (perfectION comb AG/S, Mettler Toledo) at 0, 12, 24, 48, and 72 h. Nanoparticles dispersions were prepared with a final silver concentration of 8 μ g. ml $^{-1}$. The pH of the dispersions was adjusted to 5.0, using acetic acid. Dispersions were maintained at 37°C.

Microbiology - The antimicrobial activity of Pt-AgNPs was tested against *Staphylococcus aureus* (ATCC 49230), *Pseudomonas aeruginosa* (ATCC 47085), *Escherichia coli* (extracted from rat fecal samples), and compared to that of monometallic AgNPs, PtNPs, and ionic silver. A microbroth dilution method was used, following guidelines proposed by the Clinical and Laboratory Standard Institute (Clinical and laboratory standards institute, 2017). The nanoparticles solutions were diluted to 1–80 μ g.ml $^{-1}$ and inoculated with an equal volume of bacterial suspensions in Mueller Hinton broth, which were prepared at a concentration of 10^5 CFU.ml $^{-1}$. The mixed solution was incubated at 37°C in a 5% CO $_2$ atmosphere for 36 hours. Bacterial growth curves up to 36 hours were obtained by measuring the optical density (OD) at 600 nm, using a multimode microplate reader (Tecan, Infinite™ M200 PRO). The minimum inhibitory concentration (MIC) was determined as the lowest concentration of particle solution that completely inhibited the visible growth of

bacteria ($OD_{600} = 0$). An ionic silver solution produced by electrolysis was used as a positive control, while a Mueller Hinton broth without bacterial suspension was used as a negative control.

2.2 | Statistical analysis

All experiments were conducted in triplicate. The data were expressed as mean and standard deviations. Cross-sectional diameters, aspect ratios, specific surface areas, and MICs for each experimental group were compared using the Kruskal-Wallis test combined with the modification of Tukey's adjustment for pairwise multiple comparisons as given by Conover (1999). Exact tests were used as needed. Results from ionic silver released at 72 hours were compared using one-way analysis of variance (ANOVA) combined with Tukey's adjustment for pairwise multiple comparisons. Multivariate analysis of variance (MANOVA) was used to compare the time course for ionic silver release. Relationships between level of platinum doping and MICs or ionic silver release were assessed by Spearman rank correlation. A .05 level of significance was used (Type I error level $\alpha = .05$); adjustment for multiple comparisons was made in conjunction with an overall Type I error level of .05.

3 | RESULTS

3.1 | Nanoparticle characterization

AAS analyses confirmed that silver nanoparticles were doped with platinum at the intended levels of 0.5, 1, and 2 mol% (Table 1). Mean dimensions, morphologies, aspect ratios, specific surface areas, and UV-visible spectra of doped and undoped nanoparticles are displayed in Table 1 and Figure 1a–e. There were statistically significant differences in mean cross-sectional diameter amongst all experimental groups ($p < .0001$). AgNPs appeared spherical with a localized surface plasmon resonance (LSPR) at 398 nm (Figure 1a). Pt-AgNPs exhibited mixed morphologies of spherical, oval, or ribbon-like nanostructures with decreasing mean cross-sectional diameter (Figure 1b–d). The aspect ratio of Pt-AgNPs increased as a function of platinum doping level. Although the specific surface area of Pt-AgNPs increased with the aspect ratio, there was no statistically significant difference amongst Pt-AgNPs ($p > .05$). Doping with platinum led to a blue shift of the LSPR wavelengths for Pt_{0.5}-AgNP and Pt₁-AgNPs, together with the formation of a second peak at 450 and 458 nm, respectively (Figure 1b,c). Pt₂-AgNPs exhibited only one plasmon feature at 355 nm (Figure 1d). Undoped PtNPs were spherical with a tendency for assembling as chain-like structures, while no plasmon band was observed in the UV-visible range (Figure 1e). PtNPs exhibited the highest specific surface area, compared to other nanoparticles.

Results from SERS revealed the presence of platinum dopant on the outer surface of AgNPs as indicated by the $\nu_{NC(Ag)}$ and $\nu_{NC(Pt)}$ bond stretching frequencies. Integrated band intensities were normalized using the $\nu_{CC(Ag)}$ at 1590 cm^{-1} , followed by profile fitting and deconvolution. As shown in Figure 2, platinum doping was associated with the appearance of two new bands at 2172 and $2,105\text{ cm}^{-1}$, which were assigned to the $\nu_{NC(Ag)}$ and $\nu_{NC(Pt)}$ stretching frequencies of 2,6DMPI adsorbed on platinum. By calculating the integrated intensity ratios of the $\nu_{NC(Ag)}$ and $\nu_{NC(Pt)}$ bond stretching frequencies, the amount of the

outermost surface area of nanoparticles covered by platinum was estimated at 32, 58, and 93% for Pt_{0.5}-AgNPs, Pt₁-AgNPs, and Pt₂-AgNPs, respectively.

As shown in Figure 3, the amount of ionic silver released plateaued after 24 hours. At 72 hours, the amount of ionic silver increased 16.3-fold from Pt₂-AgNPs, compared to AgNPs. The amount of ionic silver released was statistically different amongst experimental groups ($p < .0001$). The highest amount of ionic silver was released from Pt₂-AgNPs, followed by Pt₁-AgNPs, Pt_{0.5}-AgNPs and undoped AgNPs, respectively. Spearman rank correlation analysis indicated strong evidence of a dose response relationship based on the level of platinum doping ($r = .97$, $p < .0001$). Evaluation of the multivariate ionic silver release over time using MANOVA also provided strong evidence of group differences ($p < .0001$).

Microbiology - Growth curves of bacterial strains in the presence of various concentrations of nanoparticles for up to 36 h are presented in Figure 4. Doping AgNPs with platinum significantly increased the antimicrobial efficacy against all microorganisms. At equivalent concentrations, ionic silver exhibited the longest inhibitory effect (lag phase), followed by Pt₂-AgNPs, Pt₁-AgNPs, and Pt_{0.5}-AgNPs, respectively. Undoped AgNPs required at least 2.25 times the concentrations needed for Pt-AgNPs to produce the same inhibitory effect. Minimum inhibitory concentrations are presented in Figure 5. There was evidence of differences in MICs amongst the experimental groups for all three of the experimental organisms considered ($p < .0007$). Ionic silver in aqueous solution exhibited the highest antimicrobial efficacy against all bacterial strains, followed by Pt₂-AgNPs, Pt₁-AgNPs, Pt_{0.5}-AgNPs, and undoped AgNPs, in this order. PtNPs did not exhibit any antimicrobial activity at concentrations up to 80 $\mu\text{g} \cdot \text{ml}^{-1}$.

4 | DISCUSSION

Our results show that nanoparticles were successfully synthesized through the reduction of Ag⁺ and Pt²⁺ with sodium borohydride and in the presence of trisodium citrate dihydrate as a stabilizing agent. Pt-AgNPs appeared as mixed morphologies of spherical, oval and ribbon-like nanostructures which were quite similar to the Pt-Ag nanowires obtained from amine-terminated poly(N-isopropylacrylamide) (Jiang et al., 2018) or oleylamine and oleic acid as a capping agent (Peng, You, & Yang, 2010). As shown in Figure 6, functionalizing nanoparticles with bovine serum albumin (BSA) or bacterial medium allowed the preservation of in situ colloidal features (size, morphology, and dimensionality), confirming that observed morphologies of Pt-AgNPs were not altered by TEM sample preparation.

Pt-AgNPs exhibited two plasmon resonance features, which could be associated with the difference in their plasmon oscillations in the short and long axes (Hormozi-Nezhad, Jalali-Heravi, Robotjazi, & Ebrahimi-Najafabadi, 2012; Shankar, Prasad, Selvakannan, Jaiswal, & Laxman, 2015). By increasing platinum doping levels, the transverse LSPR band (355–365 nm) was blue-shifted, indicating a reduction in cross-sectional diameter. The transverse LSPR wavelength decreased linearly with the increase in aspect ratio ($R^2 = .96$, $p < .008$). Meanwhile, a red shift was observed for the longitudinal LSPR band (450–458 nm), which could be indicative of higher aspect ratios. In addition, when AgNPs were doped with 2 mol % platinum, the longitudinal LSPR extinction nearly disappeared, while the transverse

LSPR extinction band decreased in magnitude. This finding was confirmed by the results obtained from SERS, showing that platinum occupied 93% of the total surface of Pt₂-AgNPs. This is in agreement with the study by Patel et al., showing that platinum tended to cover the outermost surface of silver nanoparticles (Patel, Kapoor, Dave, & Mukherjee, 2005).

Our results show that the amount of Ag⁺ released increased with the level of doping, reaching 3.45% of the total silver content for Pt₂-AgNPs or 16.3 times greater than that of undoped AgNPs. This is in a good agreement with previous literature showing that alloying AgNPs with platinum (at least 10 mol%) enhanced the release of Ag⁺, ranging from 0.05 to 5.5% of the total silver content (Grasmik, Breisch, Loza, et al., 2018; Li & Yamauchi, 2013). The driving force for this reaction is likely derived from the difference in electrochemical potential between silver (0.7996 V) and platinum (1.18 V) (Bard, Parsons, & Jordan, 1985). In this metallic couple, silver may act as the sacrificial anode that preferentially dissolves, promoting the release of silver ions (Narozny et al., 2014). Both the amount of platinum and the chemical homogeneity of Pt on the surface of AgNPs might also play a critical role in the enhanced release of Ag⁺. Our results showed that doping AgNPs with small amounts of platinum led to the partial deposition of platinum atoms on the surface of AgNPs. The occupied surface area increased as a function of Pt doping levels. These data suggest that partial deposition of Pt on AgNPs contributed to the accessibility of electrolytic molecules, that is, oxygen, because of the oxophilic nature of silver. As a result, the electrochemically active surface of Pt-AgNPs, uniquely promoted the electrocatalytic oxidation and subsequently enhanced Ag⁺ release. In contrast, it can be expected that if the surface of AgNPs is completely occupied with platinum atoms (more than 2 mol%), the surface is relatively less oxophilic and accessibility to the Ag-Pt active sites is hindered, resulting in a decrease in the release of silver ions (Grasmik et al., 2018; Li & Yamauchi, 2013).

Previous work has shown that the MICs of AgNPs (15–20 nm) were between 90–100 µg.ml⁻¹ and 30–40 µg.ml⁻¹ when tested against *S. aureus* and *E. coli*, respectively (Agnihotri, Mukherji, & Mukherji, 2014). Our results showed slightly lower MICs. This difference could stem from variations in bacterial strains, bacterial concentrations, nutritional medium, incubation conditions or nanoparticle size (Agnihotri et al., 2014; Zheng et al., 2018). PtNPs did not exhibit antimicrobial activity in this study. This result is in agreement with previous literature for this size range (Zhao, Ye, Liu, Chen, & Jiang, 2014).

Doping silver nanoparticles with a small amount of platinum level significantly enhanced antimicrobial efficacy, lowering the MIC by up to 97% at the highest Pt-doping level of 2 mol% when tested against *P. aeruginosa*. These results are in agreement with previous literature showing that alloying AgNPs with platinum 10 mol% had an antimicrobial action against both *S. aureus* and *E. coli* with the MICs of 25 and 10 µg.ml⁻¹, respectively (Breisch et al., 2019). Our results showed that *E. coli* and *P. aeruginosa* were more susceptible to treatment with Pt-AgNPs compared to *S. aureus*. This might be attributed to the structural features of Gram-positive *S. aureus*, which are comprised of lipoteichoic acid along with thick peptidoglycan layers, decreasing the penetration of both AgNPs or Ag⁺ into the cytoplasm (Kumar, Howdle, & Münstedt, 2005). On the other hand, Gram-negative bacterial strains contain an outer membrane and a thin peptidoglycan layer (Silhavy, Kahne, &

Walker, 2010). The outer membrane is easily accessible to AgNPs or ionic silver and its disruption may explain the greater sensitivity of Gram-negative species.

From our results, ionic silver equivalent concentrations corresponding to the various MICs were calculated, leading to an antimicrobial efficacy of ionic silver against bacterial strains in the range of 0.045–0.573 $\mu\text{g.ml}^{-1}$. Although the amount of ionic silver released was approximately nine times lower than the MIC of ionic silver, Pt-AgNPs could have acted as reservoirs enabling sustained release of ionic silver. On the other hand, highly biochemically active Ag^+ could have undergone complexation with proteins or other biomolecules in media (Liu, Sonshine, Shervani, & Hurt, 2010). This would explain variations between calculated and measured values.

The amount of ionic silver released at 24 h from $\text{Pt}_2\text{-AgNPs}$ was 12.2% greater than that of undoped AgNPs. As reflected in Figure 3, there was an initial burst release of Ag^+ in the first 24 h, which was consistent with the antimicrobial efficacy of Pt-AgNPs. As shown in Figure 4, OD_{600} measurements revealed that lag phases in bacterial growth curves were prolonged compared to those of corresponding untreated bacterial species. A delayed lag phase usually indicates stress on the reproductive systems of bacteria (Silhavy et al., 2010). Therefore, we tentatively attribute the enhanced antimicrobial efficacy of Pt-AgNPs to this initial burst followed by sustained release of ionic silver (Li & Yamauchi, 2013; Zhang et al., 2016).

Another possible factor that governed the release of silver ions is the specific surface area. It was shown that $\text{Pt}_2\text{-AgNPs}$ with mixed morphologies exhibited higher specific surface areas than undoped AgNPs, which facilitated the release of Ag^+ , resulting in enhanced antimicrobial efficacy. Helmlinger, Sengstock, Groß-Heitfeld, et al. (2016), demonstrated that silver nanoplatelets exhibited the highest antimicrobial activity, followed by silver nanospheres, silver nanorods, and silver nanocubes, respectively. This antimicrobial activity directly corresponded to the dissolution rates of ionic silver, as well as the particle morphology (specific surface area). Therefore, it can be suggested that the antimicrobial efficacy of Pt-AgNPs can be ascribed to the rapidly released silver ions due to the specific surface areas associated with the morphology of the nanoparticles (Breisch et al., 2019; Chang et al., 2019).

The antimicrobial efficacy of AgNPs is well established. However, concentrations required for bactericidal activity may not be therapeutically viable and could cause localized side effects. To overcome these practical issues, silver nanoparticles doped with low levels of platinum levels (0.5, 1, and 2 mol%) were developed and tested. Our results show that the combination of two metals with different electrochemical potentials successfully triggered the sacrificial anodic effect. This promoted the generation of ionic silver, and subsequently resulted in enhanced antimicrobial activity. With their superior bactericidal efficacy, we envision that Pt-AgNPs could be deposited as coatings on the surface of dental implants in order to prevent biofilm formation. This approach could also be useful in decreasing the risk for development of antibiotic-resistant bacteria and in improving long-term clinical outcomes of dental implants. Further studies to investigate the biocompatibility of Pt-AgNPs are currently underway.

5 | CONCLUSIONS

A facile one-step method for the synthesis of platinum-doped silver nanoparticles was demonstrated. Pt-AgNPs exhibited spherical, oval, or ribbon-like nanostructures, with a cross-sectional diameter decreasing as a function of platinum doping level. The aspect ratio and corresponding specific surface area also increased with platinum doping level. The sacrificial anodic effect of platinum-doped silver nanoparticles promoted their oxidative dissolution and enhanced the release of ionic silver, which increased linearly with platinum doping levels. The lag phase in bacterial growth curves was also prolonged for Pt-AgNPs, corresponding to the initial burst followed by sustained release of ionic silver. The antimicrobial efficacy of platinum-doped silver nanoparticles increased as a function of platinum doping level and was significantly higher than that of undoped silver or platinum nanoparticles, regardless of concentration. With superior antimicrobial efficacy, Pt-AgNPs could be considered as alternative antibacterial agent to be explored for potential clinical use.

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FIGURE 1.

TEM micrographs, size distribution and surface plasmon spectra resonance of experimental groups. (a) AgNPs, (b) Pt_{0.5}-AgNPs, (c) Pt₁-AgNPs, (d) Pt₂-AgNPs, and (e) PtNPs

FIGURE 2.

Representative surface enhanced Raman spectra for 2,6-DMPI functionalized nanoparticles for each group. The stretching frequency of the isocyanide group binds to silver atoms at 2172 ($\nu_{\text{NC(Ag)}}$), 1,590 ($\nu_{\text{CC(Ag)}}$), and 645 cm⁻¹ ($\nu_{\text{C-NC(Ag)}}$), respectively. The stretching frequency of the isocyanide group binds to platinum atoms at 2105 ($\nu_{\text{NC(Pt)}}$), and 675 cm⁻¹ ($\nu_{\text{C-NC(Pt)}}$), respectively. Higher magnification of SERS spectra fitted using a Pearson VII function for (a) Pt₂-AgNPs, (b) Pt₁-AgNPs, (c) Pt_{0.5}-AgNPs, and (d) AgNPs. Inset is enlargement of the 2000–2,300 cm⁻¹ spectrum

FIGURE 3.

(a) An amount of ionic silver released as a function of time. (b) Linear relationship between amount of ionic silver released and platinum doping level

FIGURE 4. Bacterial growth curves against various nanoparticles for (a) *S. aureus*, (b) *P. aeruginosa*, and (c) *E. coli*

FIGURE 5.

Minimal inhibitory concentration of various experimental groups. No antimicrobial activity was found for platinum nanoparticles with the concentrations used in this work

FIGURE 6. Transmission electron micrographs of Pt₂-AgNPs in different media. (a) as-prepared Pt₂-AgNPs in aqueous solution, (b) serum bovine albumin-functionalized Pt₂-AgNPs, and (c) Pt₂-AgNPs in bacterial medium

TABLE 1

Dimensions and chemical composition of various experimental nanoparticles

Nanoparticles	Nanoparticles in bacterial growth conditions: Mean cross-sectional diameter (nm)	Nanoparticles in bacterial growth conditions: Mean aspect ratio (nm)	Mean specific surface area (nm ⁻¹)	Platinum content obtained from AAS (mol.%)
AgNPs	16.8 ± 3.3 ^a	1.2 ± 0.1 ^a	0.35 ± 0.09 ^a	N/A
Pt _{0.5} -AgNPs	11.2 ± 2.2 ^b	1.7 ± 0.5 ^b	0.41 ± 0.22 ^{a,b}	0.52 ± 0.09
Pt ₁ -AgNPs	10.3 ± 2.7 ^c	1.8 ± 0.7 ^{b,c}	0.45 ± 0.38 ^b	1.02 ± 0.15
Pt ₂ -AgNPs	8.4 ± 2.1 ^d	1.9 ± 0.8 ^c	0.47 ± 0.41 ^b	2.05 ± 0.14
PtNPs	7.8 ± 2.6 ^e	1.2 ± 0.1 ^a	0.80 ± 0.20 ^c	N/A

Note: Statistically significant differences were identified among nanoparticle groups with respect to the distributions of cross-sectional diameter, aspect ratio and specific surface area ($p < .0001$). Identical letters denote no statistically significant difference after multiple comparisons adjustment.