Research Article

Antibiotic delivery using gold nanoparticles

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Abstract
Antibiotic resistance is set to become one of the greatest threats to human existence and new treatments or more effective ways of treating infections have to be developed. Colistin is considered a last line of defence antibiotic which has decreased usage due to undesirable side effects and thus has reduced resistance. Due to the rise of pathogens that are resistant to all common antibacterial drugs, Colistin is once again being considered as a treatment option. As Colistin's side effects are dose dependent; it is therefore desirable to be able to treat infections using Colistin with the same therapeutic effect but at a lower dosage. Gold nanoparticles have been used as a vehicle for Colistin delivery, with a Colistin coating on both negatively charged and positively charged gold nanoparticles. This study demonstrated that by delivering Colistin on an anionic gold nanoparticle, the minimum inhibitory concentration of *E. coli* was reduced sixfold compared to antibiotic alone. The addition of Colistin coated gold nanoparticles (both positive and negatively charged) significantly reduced the growth of *E. coli* in nutrient broth over a 24 h period, with 10,000 times lower CFU per mL at 8 h compared to the control. It has also been shown that both anionic and cationic 5 nm diameter gold nanoparticles are not inherently antibacterial and do not affect bacterial growth. The anionic Colistin coated gold nanoparticles therefore show great promise for delivery of Colistin at a lower dosage with improved efficacy.

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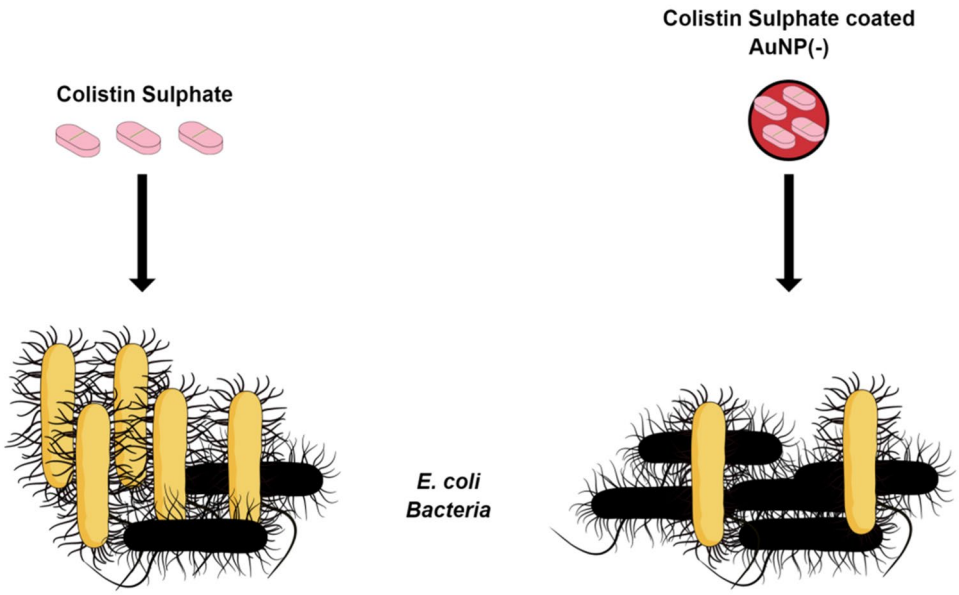
Graphic abstract



Keywords Antibiotic · Gold nanoparticles · Drug delivery · Colistin

1 Introduction

The prevalence of antibiotic resistance is quickly becoming one of the world's greatest health challenges with predictions of over 10 million deaths worldwide by 2050 [10]. Due to the global over-prescription and misuse of antibiotics, bacteria are increasingly developing resistance to common antibiotics [7]. When bacteria become resistant to multiple antibiotics, they are labelled as multi- drug resistant and infections from these bacteria are difficult and expensive to treat. Currently, these resistant bacterial infections are treated with last line antibiotics. These antibiotics are less commonly prescribed and often have higher toxicities or side effects. One of these last line drugs is Polymixin E, better known as Colistin Sulphate. Colistin's clinical use began in the 1950s; however, its use was phased out in the 1970s due to its nephrotoxic and neurotoxic side effects [8, 18]. A growing increase in the number of gram-negative pathogens that are resistant to all common antibacterial drugs has led to the reconsideration of Colistin, which, due to a lack of clinical use, is for the most part still effective at killing bacteria compared to other more common antibiotics [2, 17]. Colistin interacts with the outer membrane of gram-negative bacteria, primarily displacing calcium and magnesium ions. This increases the permeability of the membrane and therefore decreases its stability significantly, leading to the leakage of cell contents and eventual cell death [1, 8, 18].

As the side effects of Colistin are dose-dependent, it would be beneficial for the patient if a lower dose could be administered whilst still providing the same therapeutic effect [13]. It has previously been shown, that the addition of antibiotics with metal nanoparticles can lower the susceptibility of bacteria and improve the efficacy of the antibiotic [5, 14]. Therefore it is hypothesised that Colistin can be attached onto gold nanoparticles (AuNPs) and these particles can provide the same antibacterial effects at a lower Colistin dosage. Thus, this work focuses on developing a stable Colistin coated gold nanoparticle system to lower the dosage of Colistin required to inhibit bacterial growth.

Due to the low cytotoxicity, ease of functionalisation and high surface to volume ratio, AuNPs have been successfully used as drug delivery vehicles [3, 4, 12, 15, 20]. Drug-conjugated AuNPs have been investigated widely [9, 11], however antibiotic conjugation has been explored only recently. Amoxicillin coated and Kanamycin conjugated gold nanoparticles have both shown an increase in antibacterial activity compared to the antibiotic alone, suggesting the conjugation to gold nanoparticles plays a role in the mechanism of action [5, 14]. Likewise two Carbapenem antibiotics (Imipenem and Meropenem) have been conjugated through thiol bonds to AuNPs and this

attachment reduced bacterial resistance compared to the free drug whilst also improving the therapeutic activity [19].

Here, a simple electrostatic self-assembly has been utilised to attach Colistin onto citrate capped gold nanoparticles. Colistin is a cationic antibiotic, which can attach by electrostatic attraction to the negatively charged citrate capped AuNPs. With Colistin being administered in its sulphate salt form, it has the potential to destabilise the AuNP causing aggregation. Therefore, Colistin coated citrate capped gold nanoparticles (ColAu(−)) have been compared to polyelectrolyte and Colistin coated AuNPs. Due to its biocompatibility and the ability to increase stability of nanoparticle systems, Poly(diallyldimethylammonium chloride (PDADMAC) has been used as the polyelectrolyte coating [6]. PDADMAC was mixed with Colistin to fabricate PDADMAC Colistin coated gold nanoparticles (ColAu(+)) (Fig. 1).

The resultant coated gold nanoparticles (both ColAu(+) and ColAu(−)) have been characterised by UV–Vis spectroscopy and zeta potential measurements. Microbiological studies including the minimum inhibitory concentration (MIC), cell growth and cell viability assays were conducted to analyse the difference of delivering Colistin with and without gold nanoparticles.

2 Materials and methods

2.1 Fabrication of Colistin and PDADMAC coated gold nanoparticles

3 mL of 5 mg/mL PDADMAC (< 100,000 Mw) (Sigma-Aldrich, Castle Hill, Australia) in 1 mM NaCl was mixed with 200 µL of 20 mg/mL Colistin Sulphate Salt (Sigma-Aldrich, Castle Hill, Australia) and stirred for 1 h. 2 mL 5 nm diameter citrate capped gold nanoparticles (Nanocomposix, San Diego, USA) were added and mixed for a further hour. The sample was then centrifuged at 14,500 rpm for 40 min and the supernatant was removed. 1 mL of ultrapure water was added and the sample sonicated for 10 min before being washed twice more.

2.2 Fabrication of Colistin coated gold nanoparticles

200 µL of 20 mg/mL Colistin Sulphate Salt (Sigma-Aldrich, Castle Hill, Australia) was added dropwise to 2 mL of 5 nm citrate capped gold nanoparticles (Nanocomposix, San Diego, USA) and the AuNP/Colistin mixture was mixed for 1 h. The solution was centrifuged at 14,500 rpm for 40 min and the supernatant was removed. 1 mL of ultrapure water was added and the solution was sonicated for 10 min before being washed twice more.

2.3 Characterisation of Col-PDADMAC-AuNP and Col-AuNP

The size and charge of the coated gold nanoparticles was determined by UV–Vis spectroscopy (Cary-50 Spectrophotometer, Australia) and Zeta potential measurements (Malvern Zetasizer Nano). The concentration of the Colistin present on the nanoparticles was determined by UV–Vis spectroscopy at a wavelength of 219 nm. The absorbance of gold nanoparticles on their own at 219 nm was subtracted from the gold with Colistin coating absorbance reading.

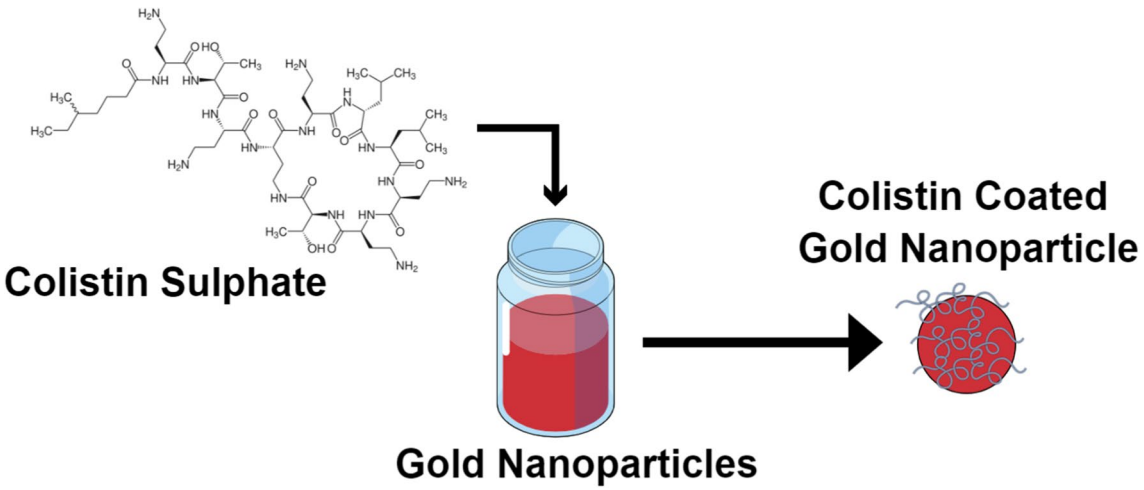


Fig. 1 Schematic of the electrostatic attachment of Colistin onto the AuNP surface

2.4 Minimum inhibitory concentration

Minimum inhibitory concentrations were determined using 96 well microplate assay. The bacterial inoculum was prepared by incubating a single colony of *Escherichia coli* American Type Culture Collection (ATCC) 700891 in nutrient broth (Sigma Aldrich, Australia) at 37 °C for 6 h with constant shaking. The cells were then centrifuged, washed and resuspended in sterile ultrapure water. The concentration was adjusted by diluting in sterile ultrapure water to an OD600 to 0.1 au. This equated to a *E. coli* concentration 10^3 CFU/mL, which was confirmed by serially diluting and drop plating 10 µl onto nutrient agar. Total CFU were counted after incubated at 37 °C for 24 h.

The 96 well microplate assay was prepared with 50 µl of nutrient broth. 50 µl of 50 µl/mL Colistin was added to the first well and then a serial 1:10 dilution was made in each subsequent well. Similarly, 50 µl of the ColAu(−) and ColAu(+) were used in subsequent rows in well 1 (see supplementary information, Table S1). In each row, well 1 contained 50 µl of Colistin or either ColAu(+) or ColAu(−), 50 µl of nutrient broth and 50 µl of bacteria at 10^3 CFU). Bacteria was not included in the negative controls. The 96 well microplates were incubated at 37 °C, overnight with constant shaking. Growth was then assessed by measuring the turbidity at OD600 using the Nanodrop Spectrophotometer (Thermo Fisher Scientific, Australia) and also by drop plating 10 µL onto nutrient agar plates (Sigma Aldrich, Australia) which were incubated overnight at 37 °C. *Escherichia coli* with no antibiotics was used as the positive control as well as the addition of AuNPs (both PDADMAC coated and citrate capped) to ensure they are not inherently antibacterial. Nutrient broth on its own was used as a negative control. All MICs were conducted in triplicate.

2.5 Effect on *E. coli* growth curve

To determine the effect of ColAu(−) or ColAu(+) on the growth curve of *E. coli* 9 mL of nutrient broth, 1 mL ColAu(+) and ColAu(−) was added with a single colony of *E. coli* (ATCC 700891). The samples were incubated at 37 °C with shaking. The growth of *E. coli* was measured every 2 h by monitoring changes in turbidity (OD600) (Nanodrop Spectrophotometer, Thermo Fisher Scientific, Australia). *Escherichia coli* in nutrient broth was used as a positive control while Colistin in nutrient broth with *E. coli* as well as nutrient broth alone were used as a negative controls. This was conducted in triplicate.

2.6 Effect on *E. coli* viability

To investigate the effect of the ColAu(−) or ColAu(+) on *E. coli* viability, single colonies of *E. coli* (ATCC 700891) were incubated in 9 mL nutrient broth at 37 °C with 1 mL of ColAu(−) or ColAu(+) for 8 h with constant shaking. This was conducted in triplicate with the following controls; nutrient broth with only *E. coli*, nutrient broth with no *E. coli*, nutrient broth with *E. coli* and a high concentration of Colistin (10 mg/mL) and nutrient broth with *E. coli* and citrate capped and PDADMAC coated AuNP. Growth was measured after 4 h and 8 h of incubation by serially diluting and drop plating 10 µL onto nutrient agar plates (Sigma Aldrich, Australia). Agar plates were incubated overnight at 37 °C and total colonies were counted. The comparison between *E. coli* concentration with the addition of ColAuNPs compared to the controls determined the effect Col coated NPs have on growth of *E. coli*.

3 Results and discussion

3.1 Confirming the attachment of Colistin

As Colistin is cationic, the molecule will attach to the negatively charged citrate capped nanoparticles through electrostatic interactions. Similarly, when Colistin is mixed with PDADMAC, they interact mainly through electrostatic interactions, however other interactions might also be present. All samples showed similar amounts of Colistin loading on the surface of the nanoparticles after three wash cycles. The ColAu(−) sample had an average of 53.3 ± 0.7 µg/mL while the ColAu(+) sample had 3.26 ± 0.5 µg/mL of Colistin (Table 1).

The presence of Colistin on the surface of the nanoparticles can be observed through UV–Vis spectrophotometry. The peak at 219 nm in a spectrum of Colistin coated AuNPs is indicative of Colistin Sulphate (see supplementary Fig. S1). The concentration of Colistin was determined using a calibration curve (see supplementary information, Fig. S2). After three wash cycles, any excess Colistin that is

Table 1 Comparison of the amount of Colistin concentration, zeta potential and AuNP absorbance peak of ColAu(+) and ColAu(+)

	Zeta potential (mV) (pH 5.9 ± 0.1)	Colistin concentration (µg/mL)	AuNP peak (nm)
AuNP(−)	−9.6 ± 8.9	0	513
ColAu(−)	6.5 ± 3.7	53.3 ± 0.7	555
ColAu(+)	35 ± 15	3.26 ± 0.5	530
PDADMAC-AuNP(+)	35 ± 8.9	0	539

not attached to the nanoparticles, is removed in the wash steps.

The addition of Colistin at the nanoparticle surface was also confirmed through the changes in zeta potential. The citrate capped gold nanoparticles had a zeta potential of -9.6 ± 8.9 mV whereas both the ColAu(+) and ColAu(−) had positively charged zeta potentials (Table 1). The change in charge indicates the attachment of cationic ligands on the particle's surface.

3.2 Stability of the nanoparticles after attachment

For a drug-delivery application, the stability of the functionalised nanoparticles is important. The ColAu(−) nanoparticle solution aggregated relatively quickly, while the ColAu(+) solutions showed a higher colloidal stability, in good agreement with previous results showing increased stability of PDADMAC-coated nanoparticles [6]. The difference in stability is evident in both the zeta potential and the UV–Vis red shift. The ColAu(−) nanoparticles had a close to zero zeta potential and red-shifted an additional 25 nm compared to the ColAu(+) sample, suggesting the formation of larger aggregates (Table 1).

The antibacterial effect of the ColAu(+) and ColAu(−) nanoparticles were tested by incubating *E. coli* with the nanoparticles. The bacterial growth curve as well as minimum inhibitory concentrations were investigated.

3.3 Antimicrobial efficacy of ColAuNPs

MICs were conducted to ascertain the effectiveness of the ColAuNPs against *E. coli*. The MIC decreased 6.8 fold between Colistin on its own and ColAu(−). There was however, no significant difference between the MIC of Colistin and ColAu(+).

Escherichia coli growth was monitored over a 24 h period using OD600 measurements. *Escherichia coli* exposed to both ColAu(+) and ColAu(−) did not show any significant bacterial growth over the 24 h period, similar to the control sample that was exposed to a significantly higher concentration of Colistin as an additional negative control (Fig. 2). The addition of Au(+) and Au(−) without the drug did not affect the growth of the bacteria, thus showing no inherent antimicrobial properties (Table 2).

While the growth curve experiments demonstrated that ColAu(+) and ColAu(−) limited the growth of *E. coli*, the cell viability assay demonstrated that the *E. coli* was still viable. The difference in concentration of *E. coli* observed in the viability assay between the Colistin coated nanoparticles and the positive control was statistically significant; suggesting that a higher Colistin concentration on the surface of the nanoparticles might be needed for complete growth inhibition. The Colistin sample on its own which was used as a negative control, contained a much greater concentration of Colistin and no bacteria was detected on drop plates over the 8 h. The ColAuNPs both were effective in significantly reducing the growth of *E. coli* compared to no treatment however it does suggest that increasing the amount of Colistin within the NP would be required to prevent all growth (Fig. 3).

The delivery of Colistin via a less stable coating, lowered the MIC of *E. coli* compared to the more stable ColAu(+) system. It could be suggested that larger aggregates of Colistin coated AuNP, are more toxic to *E. coli* compared with smaller, stable particles. This behaviour has been observed in other antibiotic-gold nanoparticle systems, where Ampicillin, Streptomycin and Kanamycin conjugated AuNPs, which all showed evidence of aggregation, had lower MICs than the respective antibiotics alone [16]. Thus, delivering Colistin as a coating on anionic gold nanoparticles is more effective at inhibiting bacterial growth compared to Colistin alone.

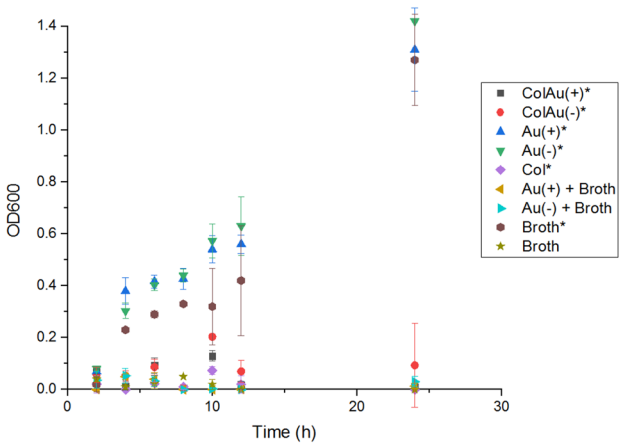


Fig. 2 Effect of ColAu(+), ColAu(−) and Colistin on the growth curve of *E. coli* (ATCC 700891) in nutrient broth measured by changes in turbidity (OD 600). *Indicates *E. coli* was present in the sample

Table 2 Minimum inhibitory concentration of Colistin, ColAu(+) and ColAu(−) against *E. coli* (ATCC 700891)

	MIC[a] (μg/mL)
Colistin	1.56 ± 0.26
ColAu(+)	1.18 ± 0.17
ColAu(−)	0.23 ± 0.03

[a]MIC is representative of the concentration of Colistin on the NPs

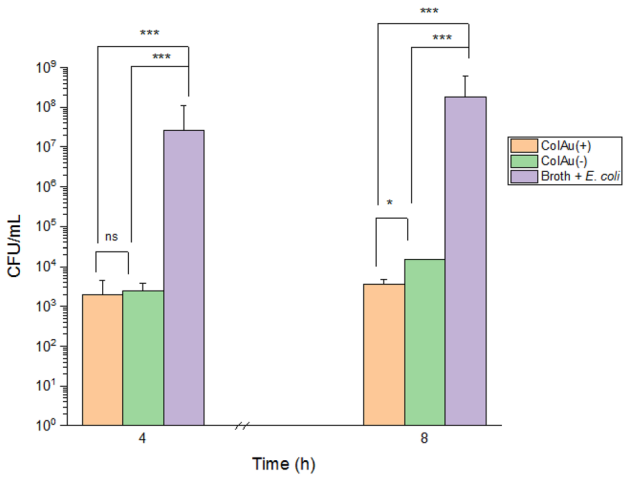


Fig. 3 Concentration of *E. coli* (CFU/mL) after 4 and 8 h of growth (at 37 °C) in nutrient broth containing ColAu(+), ColAu(−) and Colistin compared to the positive control (*E. coli* and nutrient broth). $^{*}P < 0.05$, $^{***}P < 0.0005$, ns $P > 0.05$ using one way ANOVA

4 Conclusions

This work has shown that Colistin can be easily used to coat gold nanoparticles for potential use in antibiotic delivery. The method uses a simple layer-by-layer attachment based primarily on electrostatic interactions between the negatively charged gold nanoparticles and the positively charged antibiotic, Colistin. The stability of the Colistin coated NP was improved when the cationic polymer, PDADMAC was used in the coating process. The concentration of Colistin on the surface was similar in both ColAu(+) and ColAu(−) samples and therefore the addition of PDADMAC does not considerably influence the drug-carrying load of the nanoparticles. Delivering Colistin via AuNPs showed a decrease in the MIC against *E. coli* with a 6.7 fold decrease observed for ColAu(−) compared to Colistin on its own. Therefore, a smaller dosage could be given for the same bacterial effect. AuNP on their own were shown not to affect bacterial growth and therefore are not inherently antibacterial. In addition, ColAu(+) and ColAu(−) inhibited *E. coli* growth over an 8 h time period with a 10^4 CFU per mL reduction in total growth compared to *E. coli* without the presence of an antibiotic. Overall, Colistin can be delivered via anionic AuNP with improved efficacy than Colistin in its current form, which shows potential for developing a more efficient delivery method at a lower antibiotic dosage.

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Compliance with ethical standards

Conflict of interest The authors declare there are no conflict of interest.

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