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Shape-dependent antibacterial activity of silver nanoparticles on *Escherichia coli* and *Enterococcus faecium* bacterium

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ABSTRACT

Silver nanoparticles (AgNPs) have been shown to exhibit strong antibacterial activity against both Gram-positive bacteria and Gram-negative bacteria including antibiotic resistant strains. This study aims to compare the bactericidal effect of different shaped AgNPs (spherical and truncated octahedral) against *Escherichia coli* and *Enterococcus faecium*. The antimicrobial activity of a range of concentrations (50, 100, 1000 μg/ml) was determined over 24 h using both optical density and viable counts. Truncated octahedral AgNPs (AgNOct) were found to be more active when compared with spherical AgNPs (AgNS). The difference in shape resulted in differences in efficacy which may be due to the higher surface area of AgNOct compared to AgNS, and differences in active facets and surface energies, with AgNPs having a bacteriostatic effect and AgNOct being bactericidal after 4 h. The results suggest that AgNPs can be used as effective growth inhibitors in different microorganisms, rendering them applicable to various medical devices and antimicrobial control systems.

1. Introduction

Increasing numbers of microbial organisms are becoming resistant to antibiotics demonstrating a need for new, effective antibacterial agents [1]. In this regard, nanoscale materials have emerged as potential candidates in this area due to their unique physical and chemical properties [2]. The antibacterial effects of silver (Ag) salt are well known, and have been utilised to control bacterial growth in various devices and applications; these include catheters, dentistry, burn wounds, coated medical devices and water filtration [3,4]. Moreover, a potential benefit of the use of nanoscale Ag particles (AgNPs) against microorganisms, e.g. *Escherichia coli* and *Enterococcus faecium* [5], is that such organisms are unlikely to build up resistance against AgNPs due to their broad spectrum of activity, unlike the narrow-targets of conventional antibiotics [6]. This activity includes the ability to attach through electrostatic interaction to the cell membrane and penetrate inside the bacteria, where they interact with sulphur-containing proteins and phosphorus containing compounds such as DNA [7]. Also, they have been shown to act upon the respiratory chain and replication of the bacterial cells leading to cell death [8].

The antibacterial activity of AgNps against *E. coli* and *Streptococcus mutans* has been investigated previously and found to be size dependent, demonstrating that AgNPs of 5 nm exhibit higher antibacterial activity when compared to 15 nm and 55 nm particles in Gram-positive and Gram-negative bacteria [9]. The antimicrobial efficacy of NPs can also depend on their shape. Pal et al. [5] showed truncated triangular AgNPs exhibit *E. coli* inhibition at 1 μg; however, in order for spherical NPs to inhibit *E. coli* 12.5 μg was required, with rod NPs needing a total of 50 to 100 μg of silver content [7]. In our previous work, copper nanocubes showed enhanced antibacterial activity on *E. coli* and *E. faecium* compared with copper nanospheres [10]. This study aims to determine shape dependence (spherical and truncated octahedral) on the antimicrobial efficacy of AgNP against *E. coli* and *E. faecium*.

2. Experiment

2.1. Materials

All chemicals for this work were purchased from Sigma–Aldrich, UK, without further purification. They include silver nitride ($AgNO_3$), sodium bromide (NaBr), polyvinylpyrrolidone (PVP)

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($[C_6H_9NO]_n$), ethylene glycol (EG) ($C_2H_6O_2$) and sodium citrate ($C_6H_5Na_3$).

2.2. Synthesis of AgNOct

To synthesise AgNPOct, 3 ml of two EG solutions, one containing 144 mM PVP and 0.11 mM NaBr, the other containing 94 mM $AgNO_3$, were added dropwise via a two-channel syringe pump to 5 ml of EG heated in a condenser at 160 °C. A 30 μL drop of 10 mM NaBr was then added to the pre-heated EG. The reaction solution turned yellow in a few seconds after the addition of $AgNO_3$ and PVP demonstrating the formation of AgNPs. After 10 min, the yellow colour faded in intensity due to oxidative etching and remained a light yellow colour for approximately 10 min before turning to brown and then to grey as the NPs increased in size. The synthesised NPs were then centrifuged at 4600 rpm three times and washed with de-ionised water ((DI) 18.2 MΩ MilliQ) to remove any impurities and unreacted precursors. The reactions governing the particles formation are expressed as follows [11]:

$$2HOCH_2CH_2OH \rightarrow 2CH_3CHO + 2H_2O$$

$$2Ag^+ + 2CH_3CHO \rightarrow CH_3CO\text{-}COCH_3 + 2Ag + 2H^+$$

These reactions usually occur in the presence of a mediating species such as sodium bromide, sodium sulphide, sodium borohydride and hydrochloric acid. These mediators (NaBr in this case), play a role in the etching of the particle seeds, facilitating the formation of truncated octahedrons. The PVP also works as a shape-control agent prompting the reduction of $AgNO_3$ onto specific crystal faces while preventing reduction onto others.

2.3. Synthesis of AgNS

To synthesise spherical AgNPs, an aqueous solution of $AgNO_3$ 0.001 M was heated to 100 °C and then 3 ml of sodium citrate was added. The mixture continued to be heated until the colour changed to yellow. To remove contamination and unreacted precursors from the AgNSs, a combination of centrifugation and DI water washes was used three times as before.

The particles were produced by reducing $AgNO_3$ through the following reaction [12]:

$$4Ag^+ + C_6H_5O_7Na_3 + 2H_2O \rightarrow 4Ag^0 + C_6H_5O_7H_3 + 3Na^+ + H^+ + O_2 \uparrow$$

2.4. Particle characterisation

The AgNPs of both shapes were characterised using ultraviolet/visible (UV–vis) spectroscopy (Evolution 300 UV-VIS, over the wavelength range 300 nm to 700 nm), scanning electron microscopy (SEM) and energy-dispersive X-ray (EDX) spectroscopy (LEO S430), and X-ray diffraction (XRD) Bruker D8 Advance diffractometer). The various solutions were drop-cast onto silicon and glass substrates for SEM/EDX and UV–vis/XDR investigations respectively.

2.5. Antibacterial activity studies

All investigation were carried out in triplicate on at least two separate occasions

2.5.1. Bacterial strains and culture conditions

The antibacterial activities of the AgNPs were investigated using *E. coli* (NCTC8196) and *E. faecium* (NCTC12202) as models for Gram-negative bacteria and Gram-positive bacteria respectively. Bacteria strains were stored in Luria Bertani broth at −80 °C; bacteria were then cultured in nutrient broth (NB) at 37 °C for 24 h.

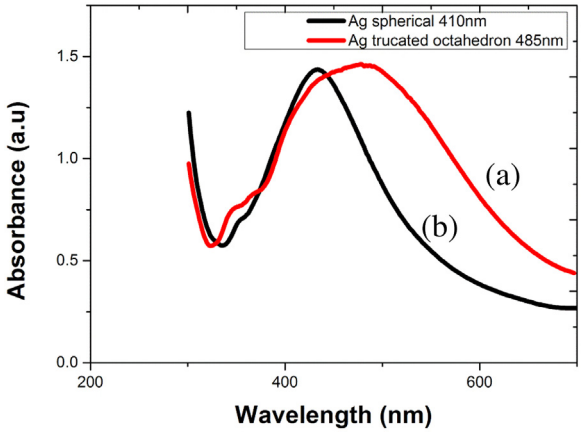

Fig. 1. UV absorption spectra (a) AgNOct and (b) AgNS.

2.5.2. Screening of AgNPs for antibacterial activity

A disc diffusion method was used to determine the antibacterial activity of both shapes of AgNPs. In brief, overnight cultures of *E. coli* and *E. faecium* were spread-plated on to nutrient agar (NA), a 2 cm paper disc was then impregnated with 50 μl of 100 μg/ml of both shapes of AgNPs and placed on the surface of the NA and incubated at 37 °C for 24 h. Zones of inhibition were then measured.

2.5.3. Determinig the growth curve of E. coli and E. faecium bacteria cells exposed to different concentration of AgNPs using optical density

To investigate the growth kinetics curves of bacterial cells exposed to AgNPs different concentrations of both shapes (1000 μg, 100 μg and 50 μg) were used. Aliquots of 200 μl of either *E. coli* or *E. faecium* in the presence of AgNPs were dispensed into 96-well plates. Optical densities (OD) were measured every hour (from 0 to 24 h) at 600 nm using a spectrophotometer (Spectra Max Plus 384). The control was wells containing bacteria only.

2.5.4. Viable count growth curves

An overnight culture of either *E. coli* or *E. faecium*, was inoculated into fresh NB (10^8) containing either AgNOct or AgNS of 1000 μg/ml. The cultures were then incubated at 37 °C in a shaking incubator and samples taken at 0, 2, 4, 6 and 24 h. Aliquots of 100 μl were spread-plated on to NA and incubated at 37 °C for 24 h. Plates were then enumerated.

3. Results and discussion

The absorption spectrum of the AgNOct solution in Fig. 1a shows an intense peak at 485 nm with shoulders at 345 nm and 365 nm. The shape and position of plasmon absorption of AgNPs are mainly dependent on dielectric medium, particle size and the surface adsorbed species [5]. Anisotropic particles such as truncated octahedrons could have two or more SPR bands depending on the shape of the particles. However, only a single SPR band is present in the absorption spectra of spherical NPs. Fig. 1b shows a sharp and intense peak at 410 nm that is attributable to the surface plasmon absorption of spherical AgNPs [13].

SEM images of prepared AgNOct and AgNS are shown in Fig. 2(a and b respectively). The AgNOct have an average diameter of ~194 nm. The size distribution is calculated from SEM images, where the polydispersity is 25% (Fig. 3a). Fig. 2b shows spherically-shaped NPs having an average diameter of ~195 nm, where the

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