Antibacterial effect of silver coated carbon nanotubes against a mucoid strain of *Pseudomonas aeruginosa*

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ABSTRACT

Pseudomonas aeruginosa is an opportunistic Gram-negative pathogen that is difficult to eradicate due to its ability to develop resistance to commonly used antibiotics. Silver coated carbon nanotubes (AgCNTs) have shown good potential in inhibiting multi-drug resistant pathogens and other food borne pathogens. Here we investigated the capacity of AgCNTs to inhibit mucoid *P. aeruginosa*. The Kirby Bauer Disk Diffusion Assay result showed that AgCNTs at concentrations of 100 µg - 400 µg, µg inhibited *P. aeruginosa* growth with clear zones of 7mm - 12 mm. Quantitative analysis of *P. aeruginosa* by colony forming units (CFU) after exposure to AgCNTs showed > 50% inhibition at 60 µg/ml by 8 hr, with little increase in inhibition by 16 hr. At 75 µg/ml of AgCNTs, inhibition was ~ 70% by 16 hr, and at 100 µg/ml inhibition was over 70% by 12 hr. Our data shows AgCNTs has antimicrobial activity against the growth of mucoid *P. aeruginosa in vitro*.

Keywords: *Pseudomonas aeruginosa*, Silver coated carbon nanotubes (AgCNTs), bacteriostatic effect, Kirby Bauer disc assay, minimum inhibitory concentration assay.

INTRODUCTION

Pseudomonas aeruginosa is a ubiquitous Gram-negative opportunistic bacterium that causes a wide array of human infections. This pathogen is a leading cause of nosocomial infections [1] wound infection [2, 3] as well as chronic lung infection in cystic fibrosis (CF) patients [4, 5]. *P. aeruginosa* is a particularly troublesome pathogen to eradicate due to its high intrinsic resistance to antibiotics such as β-lactams and fluoroquinolones. Resistance to antimicrobials in *P. aeruginosa* is largely due to the low permeability of its outer membrane and the presence of efflux pumps [6].

Adding to the inherent difficulty in treating *P. aeruginosa* infections, is the ability of the organism to convert from a non mucoid to a mucoid state. The conversion to a mucoid phenotype is followed by the deterioration and the development of antipseudomonal antibodies [7, 8]. The mucoid matrix enables the formation of protected biofilm microcolonies [9, 10, 11] and provides increased resistance to opsonization, phagocytosis, and digestion [12].

The conversion to a mucoid phenotype is a result of the synthesis of a large quantity of alginate exopolysaccharide. Although there are other virulence factors such as toxins, hemolysins, and proteases that are produced by *P. aeruginosa*, alginate production allows for consistent infections [13, 14, 15, 16], and poor prognosis for infected patients, [17, 18, 19] because this phenotype is rarely eradicated.

The identification of novel antimicrobials is needed for any microorganisms in addition to *P. aeruginosa*. One area of rapidly growing interest and study is the use of antimicrobial nanoparticles including nanomaterials coupled with metals. Silver has shown antimicrobial activity towards a wide range of micro-organisms [20]. Hence silver based nanomaterials are being used for bactericidal activity. Although the antimicrobial activity of silver is well known, its mechansim of action is not yet fully understood. Several mechanisms have been proposed such as loss in the replication ability of DNA [21]. Other studies have shown changes in membrane structure [22].

Carbon nanotubes (CNTs) have been used in many composite materials [23, 24] because of their distinct mechanical and electronic properties [25]. However, another useful property of CNTs are their antimicrobial activity, which cause physical damage to bacterial cells.[26, 27, 28]. Studies have shown that CNTs at 100 mg/ml can induced bacterial cell death by the 24 hr. Hence in this study we hypothesized that a combination of silver particles combined with carbon nanotubes (AgCNTs) would induce bacteriocidal activity against the mucoid strain, *P. aeruginosa*.

MATERIALS

AgCNTs used in this study were purchased from Nanolab, Inc. (Waltham, MA, USA). AgCNTs were dispersed in deionized water prepared with ultrapure water obtained from the Milli-O Plus system (Millipore). The tested bacteria was a mucoid strain *Pseudomonas aeruginosa* ATCC39324 (ATCC, Manassas, VA) subcultured in Todd-Hewitt (Fisher Scientific, Pittsburgh PA) broth.

METHOD

Bacterial Culture

P. aeruginosa was grown in THY and colony counts were determined by plating cultures on Tryptic soy agar (TSA) plates for quantitative analysis of antimicrobial activity.

Kirby Bauer Disc Assay

Varying concentrations of dispersed AgCNTs (100 µg, 200 µg, 300 µg and 400 µg) were added to sterile blank discs, which were then placed on seeded plates with a lawn *P. aeruginosa.* Plates were incubated overnight at 37°C.

Minimum Inhibitory Concentration (MIC)

The MIC value, an indicator of antimicrobal activity, was determined by preparing two-fold serial dilutions of AgCNTs from 0 to 400 µg/200µl. A 10^5 CFU/ml of *P. aeruginosa* were added to the different concentrations of AgCNTs in 96-well plates in Todd-Hewitt broth (THY), and then incubated at 37°C. The MIC was determined by absorbance readings taken at 600 nm every 2 hr for 8 hr and again at the 24 hr.

Quantitative analysis of Antimicrobial Activity

The antimicrobial activity of AgCNTs against the mucoid pheynotype of *P. aeruginosa* was also quantified. This was done by adding 10^4 to 10^5 CFU/ml of *P. aeruginosa* to a 5 ml culture of THY to which different concentrations of dispersed AgCNTs were also added (60 µg/ml, 75 µg/ml, 100 µg/ml and 125 µg/ml). The tubes were incubated at 37°C with shaking, followed by plating on tryptic soy agar plates at 4 hr intervals to obtain a time line study of bacterial death. The plates were then incubated at 37°C and the colonies counted the next day in order to quantify colony forming units (CFU/ml) of *P. aeruginosa.*

RESULTS

The Kirby Bauer disc diffusion assay result showed that AgCNTs at concentrations of 400 µg, 300 µg, 200 µg and 100 µg inhibited *P. aeruginosa* growth with clear inhibition zones of 12 mm, 11 mm, 10 mm and 7 mm, respectively, hence indicating inhibition of bacterial growth by all concentrations of AgCNTs (Fig. 1).

The MIC assay showed that concentrations of 100 to 400 µg/ml of AgCNTs inhibited the growth of *P. aeruginosa* as shown in Fig 2. Concentrations below 100 µg/ml increased in absorbance during the 24th hour.

Quantitative analysis of *P. aeruginosa* survival after exposure to AgCNTs showed a concentration dependent decrease in survival. By the 8th hour 60µg/ml AgCNTs was able to cause more than 50% inhibition of *P. aeruginosa's* growth. At this concentration the bacteria had a moderate increase in inhibition by the 16th hour. Concentrations of 75µg/ml and 100 µg/ml AgCNTs were able to cause 70% inhibition of growth by the 16th and 12th hours respectively (Fig 3).

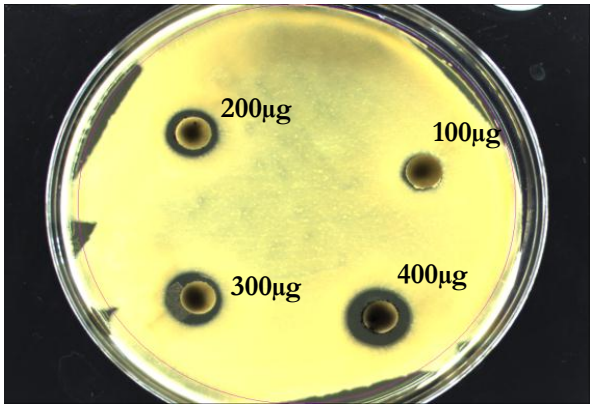

Fig 1: Kirby Bauer disc diffusionaAssay for AgCNTs against ATCC 39324. Varing concentrations of AgCNTs dispersed in water (100 µg, 200 µg, 300 µg and 400 µg) were added to sterile blank discs which was then placed on lawn of of *P. aeruginosa* on agar plates before being incubated overnight at 37°C. Clear zones aroud the discs show inhibition and were measured.

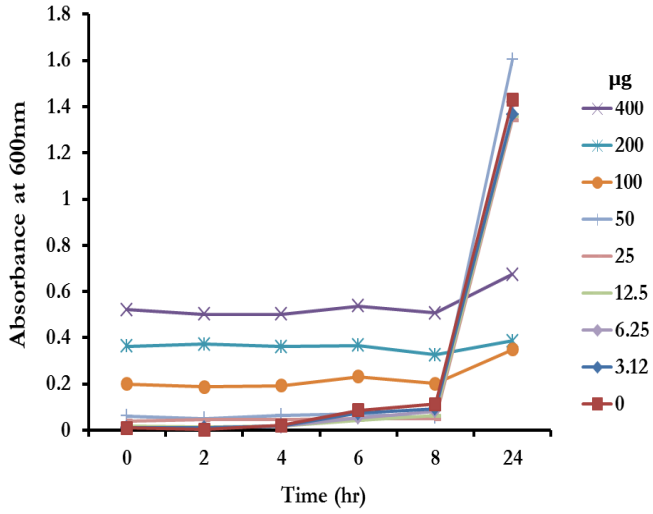

Fig 2: MIC assay. Two fold dilution of AgCNTs from 0 to 400 µg/200µl were made and 10^5 CFU/ml of *P. aeruginosa* were added to the different concentrations of AgCNTs in 96-well plates in Todd-Hewitt broth (THY) and then incubated at 37°C. MIC was determined by absorbance taken at a wavelength of 600 nm every 2 hr for up to 8 hr and again at 24 hr.

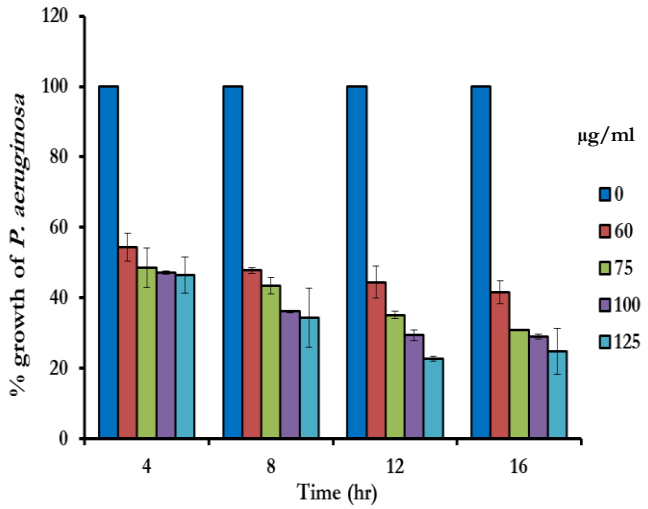

Fig 3: Quantitative analysis of antimicrobial activity. This was tested by adding 10^4 to 10^5 CFU/ml of *P. aeruginosa* to a 5 ml THY to which different concentration of AgCNTs dispersed in water were also added (60 µg/ml, 75 µg/ml, 100 µg/ml and 125 µg/ml). Tubes were incubated at 37°C with shaking. The cultures were plated on tryptic soy agar plates at 4 hr-intervals to obtain a time-line study of bacteria death. The plates were then incubated at 37°C and the colonies counted the next day in order to quantify colony forming unit (CFU/ml) of *P. aeruginosa*.

DISCUSSION

P. aeruginosa was tested with different concentrations of AgCNTs in order to observe its growth. The results demonstrated that nanoparticle could inhibit the growth of *P. aeruginosa*. Although the concentrations tested inhibited the growth of *P. aeruginosa*, significant growth of bacteria was observed only after 12 hrs, which shows that the mucoid phenotype is quite resistant to treatment by AgCNTs.

The MIC was determined by exposing *P. aeruginosa* to a two-fold dilution of AgCNTs in order to determine the minimum concentration required to inhibit *P. aeruginosa* growth for 24hr, concentrations tested did not completely inhibit the growth of *P. aeruginosa*. Various mechanisms have been proposed for antibacterial effect o nanoparticles against bacteria, DNA replication inhibiton and membrance destruction are the most commonly reported mechanisms. Thus, further research is currently being conducted to understand the killing mechansim of AgCNTs against the mucoid strain *P. aeruginosa*.

CONCLUSION

AgCNTs inhibited the growth of a mucoid strain, *P. aeruginoas* in a dose dependent fashion. This further supports AgCNTs as a probable broad spectrum antimicrobial especially in the treatment of microorganisms that are less susceptible to traditional antimicrobials.

The identification of new antimicrobial effective in the fight against *P. aeruginosa* will go far in controlling disease spread as well as decreasing the morbidity and mortality associated with *P. aeruginosa* infection.

P. aeruginosa infection is frequent and virulent in immunocompromised individuals and can spread through contact with infected surfaces, hence any anti-pseudomonad agent discovered is highly warranted. AgCNTs thus holds promise as an antimicrobial agent against mucoid *P. aeruginosa*, which is difficult to eradicate by conventional antibiotics treatment. The potential uses of AgCNTs are numerous and include being an additive in cleaning solutions and range to use in dressings of burn

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