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Antimicrobial Activity of Gold Nanoparticles and SWCNT-COOH on Viability of Pseudomonas *aeruginosa*

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Abstract

Pseudomonas aeruginosa is a prevalent, opportunistic, gram-negative bacterium that infects immunocompromised individuals, frequently causing hospital-acquired and community-acquired infections. Total of 220 specimens were collected from different clinical sites included burns, ears, wounds, urinary tract, respiratory tract.

This study found that (25) isolates belong to Pseudomonas, where six isolates (24%) were obtained from wounds, nine isolates (36%) from burns, four isolates (16%) from ear infections, two isolates (8%) from urine and four isolates (16%) from respiratory infections. And in this study the effect of gold nanoparticles and single walled carbon nanotubes on the growth had tested. Results showed that the single walled carbon nanotube has a greater effect on the growth of Pseudomonas aeruginosa after exposure to the different concentrations of the single walled carbon nanotube for 24hr and 48hr.

Introduction

Pseudomonas aeruginosa is a Gram-negative opportunistic pathogen. It is a ubiquitous bacterium that is found and isolated from various environments including plants, animals, soil, and humans. This bacterium accounts for 10-15% of the nosocomial infections worldwide and is frequently isolated from acute and chronic wound infections, respiratory infections, and the surface of medical devices (Mohamed and Abdelhamid, 2020).

Nanoparticles possess antimicrobial activity that can overcome common resistant mechanisms, including enzyme inactivation, decreased cell permeability, modification of target sites/enzymes, and increased efflux through overexpression of efflux pumps, to escape from the antibacterial activity of antimicrobial agents. NPs conjugated with antibiotics show synergistic effects against bacteria, prohibit biofilm formation, and have been utilized to combat MDROs (multidrug resistant organism). synergistic antibacterial effects of Ag, Au, and ZnO NPs and antibiotics have been observed against *S. aureus*, *E. faecium*, *E. coli*, *A. baumannii*, and *P. aeruginosa* through the penetration of the bacterial cell membrane and the interference with important molecular pathways, formulating unique antimicrobial mechanisms (Lee *et al*.,2019).

Nowadays, nanoparticles have achieved remarkable attention as novel antimicrobial products as they possess high surface area-to-volume ratio and unique physical and chemical properties. The different metals including silver, copper, titanium, zinc, and gold are used as antimicrobial materials (Liao et al ,2019).

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Gold nanoparticles (AuNPs) are used in immunochemical studies for identification of protein interactions. They are used as lab tracer in DNA fingerprinting to detect presence of DNA in a sample. They are also used for detection of aminoglycoside antibiotics like streptomycin, gentamycin and neomycin. Gold nanorods are used to detect cancer stem cells, beneficial for cancer diagnosis and for identification of different classes of bacteria (Madkour.,2018).

Carbon can bond in different ways to construct structures with completely different properties. The sp^2 hybridization of carbon builds a layered construction with weak out-of-plane bonding of the van der Waals form and strong in-plane bounds. A few to a few tens of concentric cylinders with the regular periodic interlayer spacing locate around ordinary central hollow and made MWCNTs. The real-space analysis of multiwall nanotube images has shown a range of interlayer spacing (0.34 to 0.39 nm) Depending on the number of layers, the inner diameter of MWCNTs diverges from 0.4 nm up to a few nanometers and outer diameter varies characteristically from 2 nm up to 20 to 30 nm. SWCNT diameters differ from 0.4 to 2 to 3 nm, and their length is typically of the micrometer range. SWCNTs usually can come together and form bundles (ropes). In a bundle structure, SWCNTs are hexagonally organized to form a crystal-like construction (Eatemadi *et al*.,2014).

Materials and methods

Clinical Specimens

The samples were collected from (220) patients (aged from 2 years to 55 years) who were attended to two main hospitals in Hilla city: Al-Hilla Teaching Hospital for Women and Children, and Imam Al-sadiq Hospital during a period of four months lasting from (October 2020 to January 2021). The specimens were obtained from different sites of infections (burns, wounds, ear, and urine), each swab was taken carefully from the sites of infections and transfer to the laboratory of microbiology/college of medicine. Urine (mid-stream urine) were collected from patients suffering from urinary tract infection (UTIs) in a sterile screw-cap container. Swabs from the burn wound and ear were collected from patients before they take any antibiotics or cleaning. The samples then transfer immediately to the laboratory. Each sample was streaked on MacConkey and nutrient agar and incubated aerobically at 37 Cº for 24 hours. Colonies of different morphology were isolated and bacteria were stained with gram stain and examined under the light microscope. Colonies containing bacilli were subculture on Cetrimide agar for 24 hours at 37C0. Growth and colors of bacterial colonies confirm the presence of *Pseudomonas aeruginosa.*

Preparation of SWCNT-COOH suspension

The ability to solubilize and separate discrete CNTs from their tight bundles would not only help in their use, but would also help in their purification allowing their integration in more nanobiotechnology applications (Adenuga et al., 2013).

The modification approaches, using molecules with hydrophilic and hydrophobic nature, range from single micro-molecule to complex macromolecules. Adsorption or wrapping of amphiphilic molecules, such as surfactants, onto CNTs is an efficient way to make CNTs dispersion. This modification has been done at room temperature in common solvents by

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sonication of nanotubes with surfactant. Surfactant assists CNTs dispersal in variety of solvents to form polymer-CNTs composites (Naqvi et al., 2019).
SWCNT-COOH nanoparticles in Polyethylene glycol (PEG)-400 solution (as a surfactant) to prepare a suspension of 1000µg/ml, continuous ultra-sonication(over-night) for the suspension was achieved at the time of preparation and each time prior to use so as to re-disperse the particles in the solution (Kharissova et al., 2013).

synthesis of gold nano particles

It is a chemical procedure were gold nanoparticles (AuNps) prepared as a solution, it is simple and result in aspherical gold nanoparticles which is testing by transmission electron microscopy (TEM), Scanning Electron Microscopy (SEM).

Material for synthesis Gold nano particles include:

A-Chloroauric acid

B-Trisodium citrate dehydrate (TCD) as reducing agent.

For preparing the Gold Nanoparticles a stock solution was made from Chloroauric acid and trisodium citrate dehydrate ,then to prepare this stock solution of 2 % Chloroauric acid we dissolve 1g of Chloroa ,to prepare the Gold Nano particles 2 % Chloroauric acid ,and for preparing 1% stock solution of trisodium citrate dehydrate dissolved 1g in 100 ml deionized water .After preparing the stock solution , 150µl of Chloroauric acid solution plus 50ml of deionized water must heat up to 100°C then 500µl of trisodium citrate was added (stirrer with heating at 100°C) until the clear color change into red color indicating the Gold Nano particles.

Experiments

Experiment No.1: The effect of AuNPs on Bacterial Growth at 24- and 48-hours Incubation time

Three replicate wells in four columns of 96-well plate were seeded with bacteria (*Pseudomonas aeruginosa*) in volume of 200 µl. One replicate well in column No.4 were considered as a control group, and each one of the remaining three columns three wells' replicates was exposed to 200µL of each of the serial dilutions of AuNPS (250,125,62.5,31.25,15.625, and 7.8125µg/ml). Then the plate was covered with a self-plastic lid and incubated once for 24 hours in the Incubator and the effect of AuNPS (Gold Nanoparticle) was read by ELISA reader at 405 nm. For 48 h the experiment was applied with the same conditions used in 24h experiment but the incubation period was 48 h.

Experiment No.2: The effect of SWCNT-COOH at 24- and 48-hours incubation time

Three replicate wells in four columns of 96-well plate were seeded with bacteria (*Pseudomonas aeruginosa*) in volume of 200 µl. One replicate well in column No.4 were considered as a control group, and each one of the remaining three columns three wells' replicates was exposed to 200µL of each of the serial dilutions of SWCNT-COOH (500,250,125,62.5,31.25 and 15.625µg/ml). Then the plate was covered with a self-plastic lid and incubated once for 24 hours in the Incubator and the effect of SWCNT-COOH assed by

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ELISA reader at 405 nm. For 48 hours the same conditions used in the previous experiment but after incubation time of 48h.

Result and discussion

Isolation and Identification of *Pseudomonas aeruginosa* isolates

The results indicated that 25 (11.36%) of the isolates belonged to *Pseudomonas aeruginosa*. It was found that six isolates (24%) were detected from wounds, nine isolates (36%) from burns, four isolates (16%) ear infections, two isolates (8%) from urine and four isolates (16%) from respiratory, as shown in Table below.

Table: Distribution and percentage of bacterial isolates from Different clinical specimens

Type of specimen	Total No.	No. of *P. aeruginosa* isolates	Percentage of *P. aeruginosa* isolates
Wound	50	6	24
Burn	55	9	36
Ear	45	4	16
Urin	30	2	8
Respiratory	40	4	16
Total	220	25	100

These results indicate that *Pseudomonas aeruginosa* was highly isolated from burn infection (36%) which is comparable to (Bekele et al.,2015) he found that Pseudomonas aeruginosa was isolated in 49.32% catheterized patients in Ethiopia. While (Adeyemi et al.,2020) found 61 (25.7%) isolates of *Pseudomonas aeruginosa* were recovered from 237 wound samples from the two hospitals selected for this study.

The effect of Gold Nanoparticles and SWCNT-COOH on viability of *Pseudomonas aeruginosa*

Results showed that there was a highly significant ($p \leq 0.001$) decrease in growth of bacteria after exposure to the different concentrations of gold nanoparticles and SWCNT-COOH for 24hr and 48hr. as expressed in **Figure (A, B)**

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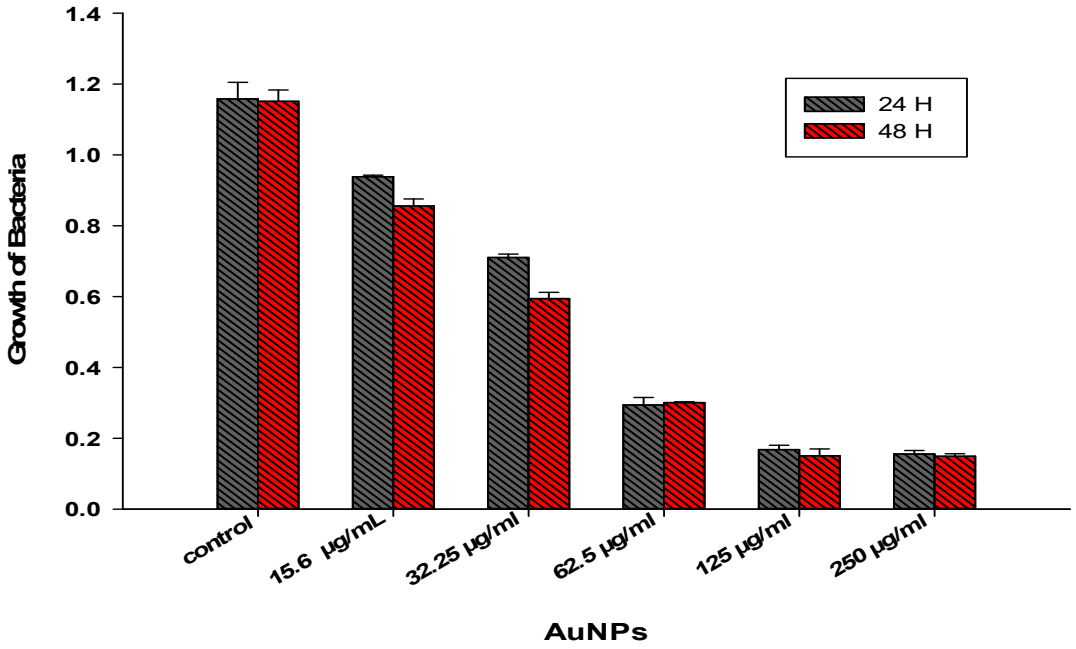

Figure A: The effect of different concentration of AuNPs on *Pseudomonas aeruginosa*

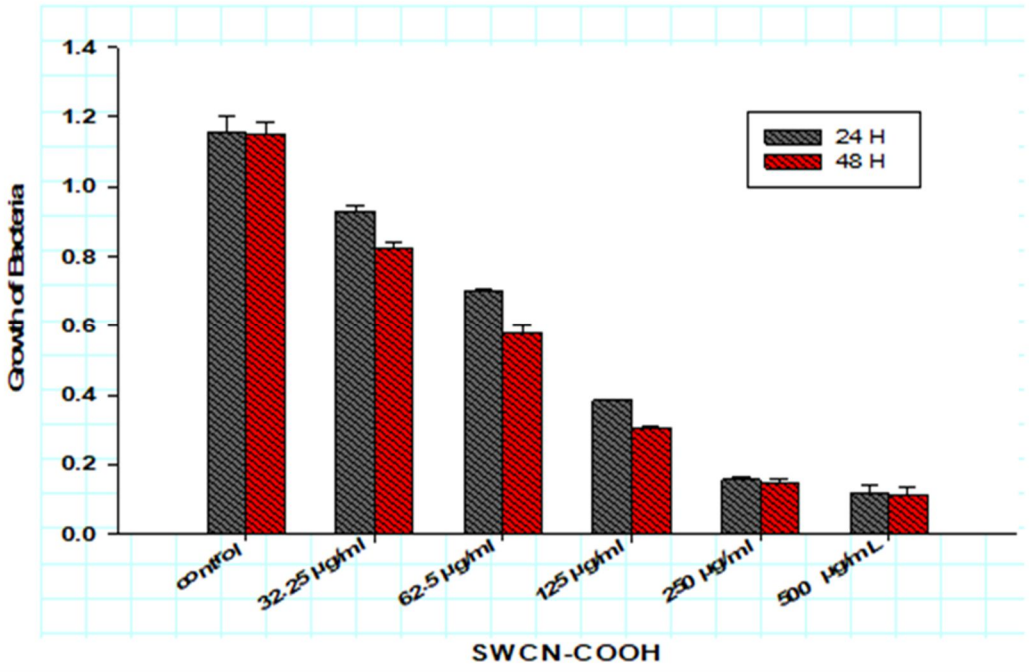

Figure B: The Effect of different concentration of SWCN-COOH on *Pseudomonas aeruginosa*

The decrease in the growth of bacteria may be due to uptake of single gold nanoparticles by bacteria and rearrangement of them inside cytoplasm (Zhou *et al*.,2012). The small sizes of AuNPs help to penetrate bacterial cells (Minai *et al*.,2013). Also, AuNPs can interact with

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cell membrane of bacteria, resulting in formation of distinct aggregation patterns and lysis of bacterial cell (Li *et al*.,2014).

The antibiotic resistance of *Pseudomonas aeruginosa is* primarily due to the co-operation of multidrug efflux pumps and impermeability of bacterial membrane. Therefore, it is possible that Au3+ may sensitize *Pseudomonas aeruginosa* by either interfering in the function of these efflux pumps or increasing the permeability of the bacterial membrane (Nazari *et al*.,2012).

For SWCN the cytotoxicity has concluded to membrane stress (i.e., direct SWNT−bacteria contact resulting in membrane perturbation and the release of intracellular contents) was the primary cause of cell death because Carbon nanoparticles might cross the cell membranes, penetrating into the interior of the cell and interacting with intracellular sites, by preventing bacteria from dividing and multiplying. It induces cell lysis and kills the bacteria (Varghese *et al*.,2013)

Gene expression studies have indicated oxidative stress may be active, as well. Here, loss of bacteria viability was observed to increase with an increasing concentration of SWCN. Previous study indicates that SWCN toxicity mechanism by in vitro SWCN-mediated oxidation of glutathione, a common intracellular thiol that serves as an antioxidant and redox state mediator. The extent of glutathione oxidation was observed to increase with increasing fraction of metallic SWNTs, indicating an elevated role of oxidative stress. The decrease in the bacteria growth may be due to the three-step SWNT antimicrobial mechanism involving (i) initial SWNT−bacteria contact, (ii) perturbation of the cell membrane, and (iii) electronic structure-dependent bacterial oxidation (Vecitis *et al*.,2010).

Conclusion

AuNPs and SWCNT-COOH have been found to have a vital and effective revolution for drug delivery. Also, they perform as a non-dangerous and non-toxic antimicrobial agent refers to their functional effective nature when compared with antibiotics.

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