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Research Article

EFFECT OF SILVER NANOPARTICLES ON THE GROWTH OF BACTERIAL ISOLATES FROM URINARY CATHETERS AND INTRAUTERINE DEVICES

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Abstract

The mechanism of prevention of bacterial growth by antibiotics is quite different from the mechanisms by which nanoparticles inhibit microbial growth. But in the present study effect of silver nanoparticles on the isolates from catheter and intra uterine devices biofilms was studied. Also *invitro* studies were carried out to find microbial biofilm formation on catheter tips and IUDs. Silver nanoparticles in aqueous solution were prepared by reduction of $AgNO_3$ with $NaBH_4$ and stabilized by using tri sodium citrate. The nanoparticles obtained hereby are stable for weeks. Microorganisms were isolated and were sub cultured in Muller Hinton broth for 24h at 37^{o}C. Wells of 6mm diameter were made on Muller Hinton agar plates using gel puncher. This study shows that the biofilm isolate *P. mirabilis* is very sensitivity to silver nanoparticles than standard antibiotics. The sensitivity of *Neisseria gonnorrhaea* to silver nanoparticles is little less. This can be seen from the diameter of zone of inhibition. So infections in patient with individually catheter and IUDs are difficult to treat with high dose of antibiotics. As silver nanoparticles even at a small dose inhibit the bacterial colonisation these particles can be coated on the devices to inhibitory bactericidal concentration.

Keywords: Intrauterine devices (IUDs), Copper-T and cervical swab, Cystine lactose electrolyte deficient agar medium (CLED), microtitre plate (MTP), SEM, *Proteus mirabilis.*

Introduction

In the present scenario, nanoscale materials have emerged up as novel antimicrobial agents owing to their high surface area to volume ratio and the unique chemical and physical properties (Morones *et al*., 2005; Kim *et al*., 2007). One of the most studied aspects of nanotechnology nowadays is their ability to offer the opportunity to fight microbial infections via synthesis of nanoparticles (Cho *et al.*, 2005). The mechanism of prevention of bacterial growth by antibiotics is quite different from the mechanisms by which nanoparticles inhibit microbial growth. Therefore, nanoparticles have the potential to serve as an alternative to antibiotics and to control microbial infections such as those caused by MRSA (Cho *et al.*, 2005).

Silver has been used for thousands of years as a precious metal by humans in different applications as jewellery, tools, coins, photographic material or explosives. Hippocrates described the use of silver powder for its application in wound healing and in the treatment of ulcers (Klasen[a], 2000). In the 17th and 18th centuries, silver nitrate was used for ulcer treatment and its antimicrobial activity was established in the 19th century. Nevertheless, after the introduction of the antibiotics in 1940 the use of silver salts decreased. Subsequently, silver salts and silver compounds have been used in different biomedical fields, especially in burn treatment (Klasen[b], 2000).

The antimicrobial activity of Silver nanoparticles (Ag-NPs) appears significantly high. Silver is more toxic element to microorganisms than many other metals in the following sequence: Ag > Hg > Cu > Cd > Cr > Pb > Co > Au > Zn > Fe > Mn > Mo > Sn (Zhao and Stevens, 1998). Silver nanoparticles exert more efficient than silver ions and other silver salts in mediating their antimicrobial activity (Lok *et al*., 2006; Rai *et al*., 2009). Silver exhibits low toxicity to mammalian cells (Zhao and Stevens 1998). Silver has a lower propensity to induce microbial resistance than many other antimicrobial materials (Kim *et al*., 2007 and Silver *et al*., 2006). As a result, Silver nanoparticles have been applied to a wide range of products, the most important current use is as antimicrobial agents to prevent infection, such as in burn and traumatic wound dressings, diabetic ulcers, coating of catheters, dental works, scaffold, and medical devices (Silver *et al*., 2006; Kim *et al*., 2007; Rai *et al.,* 2009). Silver nanoparticles are also used in hygienic products including water purification systems, linings of washing machine, dishwashers, refrigerators, and toilet seats (Silver *et al*., 2006; Rai *et al*., 2009).

Nanosilver is one of non-toxic and safe antibacterial agents to the human body. Besides, silver nanoparticles are also reported to possess anti fungal activity (Kim *et al*., 2007), anti-inflammatory effect (Nadworny *et al*., 2008), anti viral activity (Rogers Parkinson *et al*., 2008) and anti angiogenic activity (Kalishwaralal *et al*., 2009). But, silver nanoparticles can be well applied in therapy safely when the effective concentrations of silver nanoparticles on various types of organisms are determined; Hence Silver nanoparticles ability to inhibit growth of microbes in *invitro* and *invivo* conditions was studied.

In the present study *Proteus mirabilis, Pseudomonas aeruginosa, Escherichia coli, Neisseria gonnorrhaea, Staphylococcus aureus, Staphylococcus epidermidis and Candida albicans* were isolated from the catheters and IDUs tested. In order to present the growth of biofilm formation on medical implants several methodologies may be used. But in the present study effect of silver nanoparticles on the isolates from catheter and intra uterine devices biofilms was studied. Also *invitro* studies were carried out to find microbial biofilm formation on catheter tips and IUDs.

Materials and Methods

Preparation of silver nanoparticles

Silver nanoparticles in aqueous solution were prepared by reduction of $AgNO_3$ with $NaBH_4$ and stabilized by using tri sodium citrate. All reagents used in the synthesis were of analytical grade and solutions were prepared with Milli Q water (18 MX cm). Briefly, 1ml of a 5mM aqueous $AgNO_3$ solution was added to 16 ml of a 1.06mM aqueous sodium citrate solution under magnetic stirring in an ice/water bath at around 0^oC. Then, 100µl of a freshly prepared 100mM aqueous $NaBH_4$ solution were added drop wise over 5min. The initially colourless solution became yellow and was stirred at around 0^oC for 1h and 45min. The final Silver concentration in the nanoparticles solution is 3.16×10^{-2}mg Ag/ml (50mm size). The nanoparticles obtained hereby are stable for weeks.

Bacterial cultures

Seven bacterial strains isolated from the urinary catheters and IUDs viz; *Proteus mirabilis, Pseudomonas aeruginosa, Escherichia coli, Neisseria gonnorrhaea, Staphylococcus aureus, Staphylococcus epidermidis and Candida albicans* were used.

Antibiogram

Proteus mirabilis, Pseudomonas aeruginosa, Escherichia coli, Neisseria gonnorrhaea, Staphylococcus aureus, Staphylococcus epidermidis and Candida albicans were isolated and were sub cultured in Muller Hinton broth for 24h at 37^oC. Wells of 6mm diameter were made on Muller Hinton agar plates using gel puncher. Each strain was swabbed uniformly into the individual plates using sterile cotton swabs. Using sterile micropipette 0.002µ**g** of the sample of nanoparticles solution (50 nm particle sizes) was poured onto each of the wells at the centre in all the plates. After incubation at 35^oC for 24hr, the different levels of zone inhibition were measured using Hi- media antibiotic zone scales. The diameter of zone of inhibition was compared with the zone obtained for a standard antibiotic Ciprofloxacin (Table 1).

Results and Discussion

Sensitivity to silver nanoparticles treatment

Silver nanoparticles sensitivity assay showed that all the bacterial isolates were highly sensitivity. Of the different bacteria isolated *P. mirabilis* showed the maximum sensitivity (zone diameter 7.3±0.3mm). But the same isolates showed an inhibition zone of 11.5mm diameter for antibiotic Ciprofloxacin. This study shows that the biofilm isolate *P. mirabilis* is very sensitivity to silver nanoparticles than standard antibiotics. The sensitivity of *Neisseria gonnorrhaea* to silver nanoparticles is little less. This can be seen from the diameter of zone of inhibition (Table 1).

Table: 1 Inhibitory effects of silver nanoparticles (SNP) (50nm size) on the bacterial isolates from urinary catheter and intrauterine devices

S. No	Microorganism	Diameter zone of inhibition (in mm)	Ciprofloxacin
		SNP	
1.	*Proteus mirabilis*	18.2±0.2mm	7.3±0.3mm
2.	*Pseudomonas aeruginosa*	16±0.9mm	12±0.7mm
3.	*Escherichia coli*	16.3±0.7mm	13.5±0.8mm
4.	*Neisseria gonnorrhaea*	17.5±1.2mm	13.6±0.6mm
5.	*Staphylococcus epidermidis*	16.8±1.5mm	14±0.6mm
6.	*Staphylococcus aureus*	16.4±0.6mm	13±0.4mm
7.	*Candida albicans*	16.3±0.5mm	12.5±0.6mm

± = Values are given as mean SD. (n=5)

Minimum inhibitory concentration

The sensitivity of silver nanoparticles for bacterial isolates from urinary catheter and IUDs to were tested and compared with standard antibiotics it was observed that all the bacterial isolates from the catheters were sensitive to the silver nanoparticles. The diameters of zone of inhibition developed in the clinical isolates were compared with that of standard laboratory culture. The results indicate that the bacteria isolated from urinary catheters were sensitive to silver nanoparticles but resistant to standard antibiotics. So infections in patient with individually catheter and IUDs are difficult to treat with high dose of antibiotics. As silver nanoparticles even at a small dose inhibit the bacterial colonisation these particles can be coated on the devices to inhibitory bactericidal concentration.

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