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Anti Biofilm Effect of Biogenic Silver Nanoparticles Coated Medical Devices against Biofilm of Clinical Isolate of Staphylococcus Aureus

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Abstract - Biofilm represents the most prevalent type of virulent factor of most of the pathogenic microorganism and involved in crucial development of clinical infection and exhibit resistance to antimicrobial agents. Now the biofilm is considered as major target for the pharmacological development of drugs. A biofilm serves to promote bacteria persistence by resisting antibiotic treatment and host immune responses. Antibiotics are rendered ineffective when biofilms form due to their relative impermeability, the variable physiological status of microorganisms, subpopulations of persistent strains, and variations of phenotypes present. Metal nanotechnology chemistry has the potential to prevent the formation of these life-threatening biofilms on life supporting devices. In the present study, anti biofilm effect of silver nanoparticles coated catheter against clinical isolate of Staphylococcus Aureus was studied. Silver nanoparticles synthesized by leaf extract broth of Azadirhacta indica were coated on the catheter chara-cterized by scanning electron microscopy which reveals complete dispersion of the nanoparticles on the fibre surface of the catheter and the size, shape of the particles shows uniform spherical particles with the size of 50-60 nm. Distinct effect of biofilm inhibition was recorded in the nanoparticles coated catheter and maximum inhibition was observed during 72 hour of incubation. Biochemical composition of biofilm matrix mainly total carbohydrates and total protein was highly reduced. The present study would suggests the development of anti microbial coated medical devices against pathogenic microorganism.

Keywords : *biogenic silver nanoparticles, biofilm, catheter.*

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Anti Biofilm Effect of Biogenic Silver Nanoparticles Coated Medical Devices against Biofilm of Clinical Isolate of *Staphylococcus Aureus*

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Abstract - Biofilm represents the most prevalent type of virulent factor of most of the pathogenic microorganism and involved in crucial development of clinical infection and exhibit resistance to antimicrobial agents. Now the biofilm is considered as major target for the pharmacological development of drugs. A biofilm serves to promote bacteria persistence by resisting antibiotic treatment and host immune responses. Antibiotics are rendered ineffective when biofilms form due to their relative impermeability, the variable physiological status of microorganisms, subpopulations of persistent strains, and variations of phenotypes present .Metal nanotechnology chemistry has the potential to prevent the formation of these life-threatening biofilms on life supporting devices. In the present study, anti biofilm effect of silver nanoparticles coated catheter against clinical isolate of Staphylococcus *aureus* was studied. Silver nanoparticles synthesized by leaf extract broth of *Azadirhacta indica* were coated on the catheter chara-cterized by scanning electron microscopy which reveals complete dispersion of the nanoparticles on the fibre surface of the catheter and the size, shape of the particles shows uniform spherical particles with the size of 50-60 nm. Distinct effect of biofilm inhibition was recorded in the nanoparticles coated catheter and maximum inhibition was observed during 72 hour of incubation. Biochemical composition of biofilm matrix mainly total carbohydrates and total protein was highly reduced. The present study would suggests the development of anti microbial coated medical devices against pathogenic microorganism.

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I. Introduction

Biofilms are universal, complex, interdependent communities of surface associated micro-organisms. The organisms are enclosed in an exopolysaccharide matrix occurring on any surface, particularly aquatic and industrial water systems as well as medical devices. As such, biofilms are highly relevant for public health (Donlan and Costerton, 2002). Biofilm, likely the predominant mode of device related microbial infection exhibit resistance to antimicrobial agents (Adonizio *et al.*, 2008). They can serve as hides for disease and are often associated with high level antimicrobial resistance of the associated organisms. Biofilms create an environment that enhances antimicrobial resistance. The EPSs of biofilms contain considerable amounts of polysaccharides, proteins, nucleic acids and lipids which are responsible for maintaining structural integrity of the biofilm and provide an ideal matrix for bacterial cell growth. Intermolecular interactions between the functional groups within these macromolecules serve to strengthen the overall mechanical stability of the EPSs and the survivability of the microorganisms..During the past 20 years it has been reported that between 6 and 14% of patients that enter general hospitals develop a nosocomial infection (Vazquez-Argon *et al.*, 2003), i.e., an infection that was not present or incubating at the moment of patient admission at a hospital. Over-all, a large percentage of biofilm-related infections are associated with indwelling medical devices: about 1 million cases- an estimated 60% of nosocomial infections are due to biofilms that have formed on indwelling devices (Darouiche, 2004). Medical imaplants that are more prone to biofilm formation include: artificial voice prostheses, replacement joints, prosthetic heart valves, cardiac pacemakers cerebrospinal fluid shunts, endotracheal tubes, urinary catheters, contact lenses, dental implants (Hall-Stoodley *et al.*, 2004; von Eiff *et al.*, 2005). When a biofilm infection occurs, the microorganism establishes an immune response to antigens released from the biofilm.

The main microorganisms responsible for biofilm formation on indwelling medical devices are: Gram-positive (*Enterococcus faecalis*, *Staphylococcus aureus*, *Staphylococcus epidermidis*, and *Streptococcus viridans*) and Gram-negative (*Escherichia coli*, *Klebsiella pneumoniae*, *Proteus mirabilis*, *Pseudomonas aeruginosa*) bacteria as well as yeasts (Davey and OToole, 2000). *Candida* species are in fact emerging as important nosocomial pathogens, and approximately 80% of patients with candidemia possess a CVC, a fact

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which highlights the importance of yeasts from *Candida* spp., as common causes of CVC related infections (Ben-Ami *et al.*, 2008). Inhibition of biofilm is considered as drug target and the pharmacological inhibition of biofilm development is now extensively studied for the treatment of various bacterial and fungal infections (Karthick Raja Namasivayam and Allen Roy, 2013). In the present study, anti biofilm effect of biogenic silver nanoparticles coated catheter against clinical isolate of Staphylococcus aureus has been discussed.

II. Materials and Methods

a) Synthesis of Biogenic Silver Nanoparticles

Biogenic silver nanoparticle was synthesized from leaf extract broth of *Azadiracta indica* (neem) 100gm of dried leaf material was homogenized finely in domestic mixer and 1gm of homogenized material was dissolved in 100ml deionised water and filtered through crude filter paper 50ml of collected filtrate was transferred to 100 ml of beaker and 100ml of 0.1mM silver nitrate was added and the preparation was kept under magnetic stirrer. Conversion of reaction mixture from pale green to dark brown indicates synthesis of silver nanoparticle and further conformation was carried out by uv- visible spectroscopy, Scanning Electron Microscopy and Energy Dispersive Atomic spectroscopy (EDX) the characterized particle was used for further studies.

b) Bacterial Stains

Clinical isolate of *Staphylococcus aureus* was obtained from Madurai medical college hospital, Tamil Nadu, India. Bacterial strain was maintained on slope of nutrient agar slant. (Hi media, Mumbai). Nutrient broth was used for inocula preparation. Cultures was inoculated from fresh slopes and incubated with shaking at 37^0C for 24 hours. Cells were collected by centrifugation and the collected cell debris washed twice in phosphate buffer saline and suspended to OD520 prior to use in biofilm experiments.

c) Biofilm Inhibition Assay

Biofilm inhibition carried out in 96 well plates adopting modified method of biofilm spectrophotometric assay (Toole and Kolter, 1998).100µL of cell suspension of the strain thus prepared was added in to 96 well time plate and different concentration of nano particle added and incubated at 37^{o}c for three days after the incubation the liquid culture was removed and 100µL of 1% weight/ volume aques solution of crystal violet was added. Following staining at room temperature for 30 minutes the dye was removed and wells were washed thoroughly, 95% ethanol was added and incubates for 15 minutes the reaction mixture was read spectrophotometrically at 590 nm. Biofilm inhibition (%) was calculated by the following formula

$$\% \text{ of inhibition} = \frac{\text{OD in control} - \text{OD in treatment}}{\text{OD in control}} \text{x100}$$

Catheter was obtained from local medical shop (romo10) the catheter was cut in to 1x1 cm^2 surface and the cut pieces (5 nos) were transferred to a beaker containing 20mL of silver nanoparticles suspension with 100µg concentration kept in ultrasonicator for three hours at room temperature, Coating of nanoparticles was confirmed by color change of the catheter surface fine dispersion of particle by scanning electron microscopy and Fontier transform infra red spectroscopy (FTIR) these pieces were used for biofilm inhibition study.

d) Biofilm Inhibition Study

The cut pieces was transferred to a test tube containing 5mL of 24 hour culture, the inoculated tubes were kept in 37^0C for 3 days (72 hrs) after the incubation period the whole content was aspirated and 5mL of 1% crystal violet was added and incubated at room temperature for 10mins. Crystal violet was removed and successive washing was made using sterile phosphate buffer saline to remove unbound cells or free plantonic cells. After washing, 5mL of ethanol was added kept at room temperature for 15 minutes the reaction mixture was read at 590 nm and the biofilm inhibition was determined as described earlier.

III. Evaluation of Biochemical Composition of Biofilm Matrix

a) Isolation of Biofilm Matrix

Isolation of biofilm matrix material from the microtitre plate and catheter was carried out by standard method. Adherent biofilms were transferred to screw cap bottles containing 10 ml distilled water. The bottles were sonicated for 5 min in an ultrasonic water bath and vortexed vigorously for 1 min to disrupt the biofilms. Cell suspensions were then pooled and centrifuged. The collected supernatant used as source for studying biochemical composition mainly protein by Lowry *et al* and total carbohydrate by Dubois *et al.*

IV. Result and Discussion

Biogenesis of silver nanoparticle from leaf extract broth of *Azadiracta indica* was primarily confirmed by colour change of the reaction mixture from green to brown, plasmon absorption maxima at 420nm by U.V spectrophotometer (Figure 1).Particles morphology was studied by Scanning electron microscopy (SEM).SEM images were recorded by using a Carlzeiss Supra 55 field emission scanning electron microscope equipped with an energy-dispersive spectrum (EDS, oxford instruments) capability. In a SEM setup, the nanoparticulate sample, coated to be conductive (e.g.

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gold, palladium), is scanned in a high vacuum chamber with a focused electron beam. The scanning electron microscopy study reveals uniform spherical particles with the size of 50-60nm and the presence of silver in the reaction mixture was further confirmed by EDAS.

Biofilm inhibition study clearly revealed all the tested concentration inhibited biofilm of *Staphylococcus aureus* .Results were represented as inhibition percentage of biofilm development (Table 1). In microtitre plate assay, anti biofilm effect was observed as dose dependent manner. As presented in table 1, silver nanoparticles with 100μg/ml recorded maximum anti biofilm effect with 84.0 followed by 75.5, 69.0, 59.4 and 42.1 % inhibition at the respective concentration.

Coating of biogenic silver nanoparticle was easily identified by color change of catheter (Figure 3) dispersion of nanoparticle on the catheter surface was confirmed by scanning electron microscope which reveals the uniform spherical particles were embedded on the catheter surface with the size of 50 to 60nm (Figure 4). Frontier transform infra red spectroscopy (FTIR) reveals the characteristic changes in the vibrational peaks of coated and non coated catheter (Figure 5). Biofilm inhibition study revealed 87.0 % inhibition during 72 hours of incubation period. Surface topography with SEM reveals complete degeneration of biofilm with weakened cell masses (Figure 6). Similar anti biofilm effect of chemogenic silver nanoparticles coated catheter against clinical isolate of *Staphylococcus aureus* has been reported (Karthick Raja Namasivayam *et al*, 2012). Biochemical composition of biofilm matrix total carbohydrate and total protein was also highly reduced. The matrix is one of the most distinctive features of a microbial biofilm. It forms a three dimensional, gel- like, highly hydrated and locally charged environment in which the microorganisms are largely immobilized. Matrix-enclosed micro colonies, sometimes described as stacks or towers, are separated by water channels which provide a mechanism for nutrient circulation within the biofilm the composition of the matrix varies according to the nature of the organism and reduction of the biochemical composition of the biofilm matrix leads to weakening of the biofilm thus facilitate entry of the drugs. In respective concentration of nanoparticles treatment, 70.0, 57.0, 31.9,14.5 and 8.5 μg of total carbohydrates was recorded under microtitre plate assay Similarly,79.0, 45.0,30.0, 22.0 and 17.5 μg of protein were recorded Similar reduction of carbohydrate as 5.0 and 13.0 μg of protein was observed in nanoparticle coated catheter (Table 2).

V. Acknowledgement

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Figure 1 : UV vis spectra of synthesized silver nanoparticles

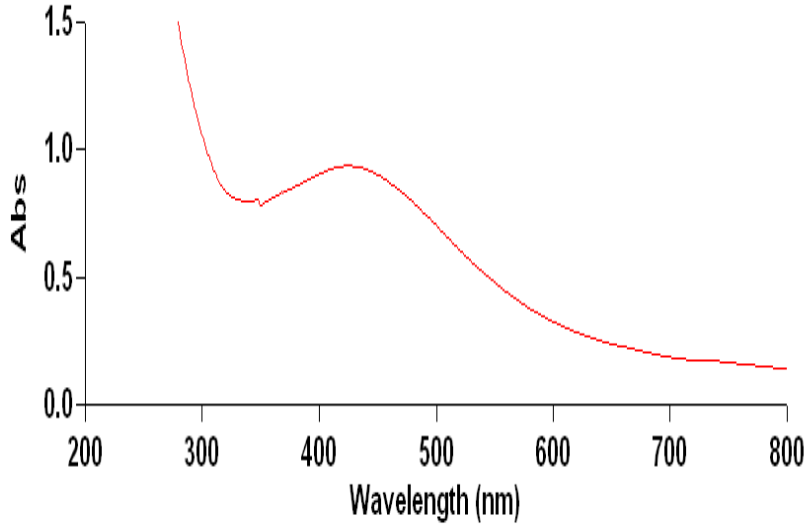


Figure 2 : SEM image of synthesized nanoparticles

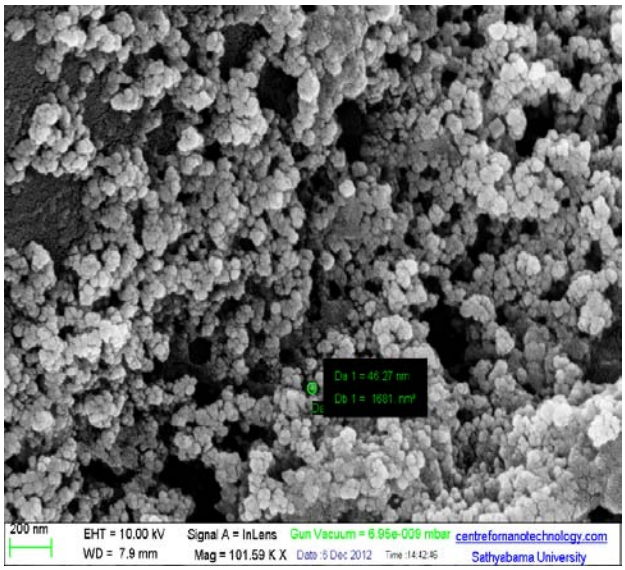


Figure 3 : Silver nanoparticles coated catheter



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Figure 4 : FTIR spectra of un coated coated catheter

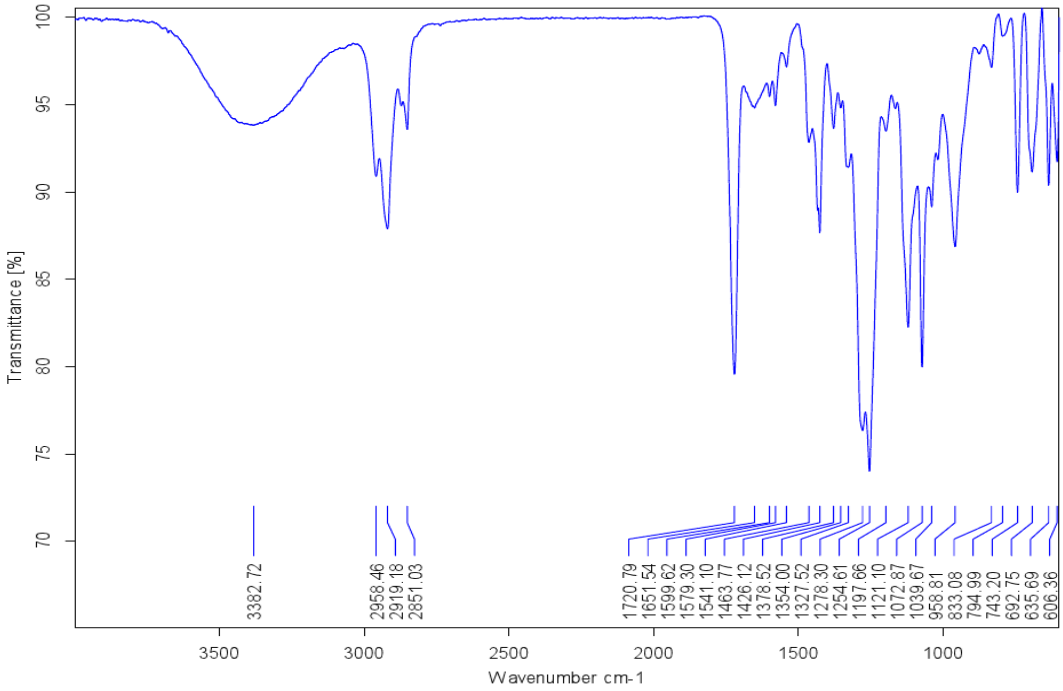
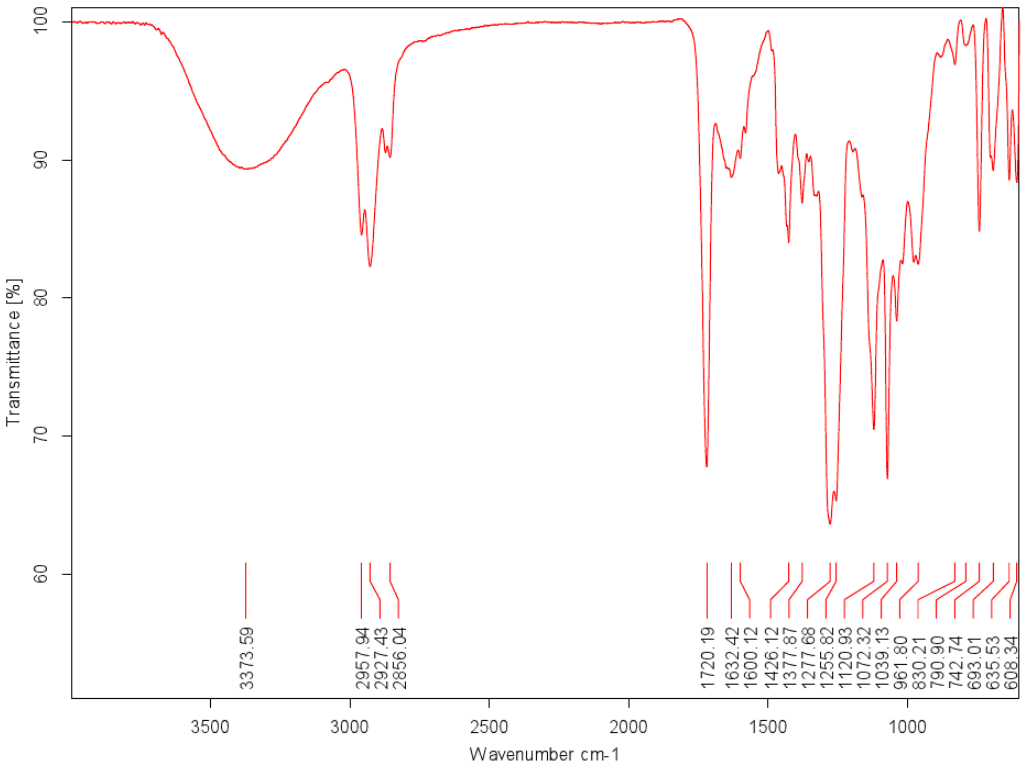


Figure 5 : FTIR spectra of silver nanoparticles coated catheter



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Figure 6 : SEM image of loose cell distrupted *Staph.aureus* biofilm on the silver nanoparticles coated catheter

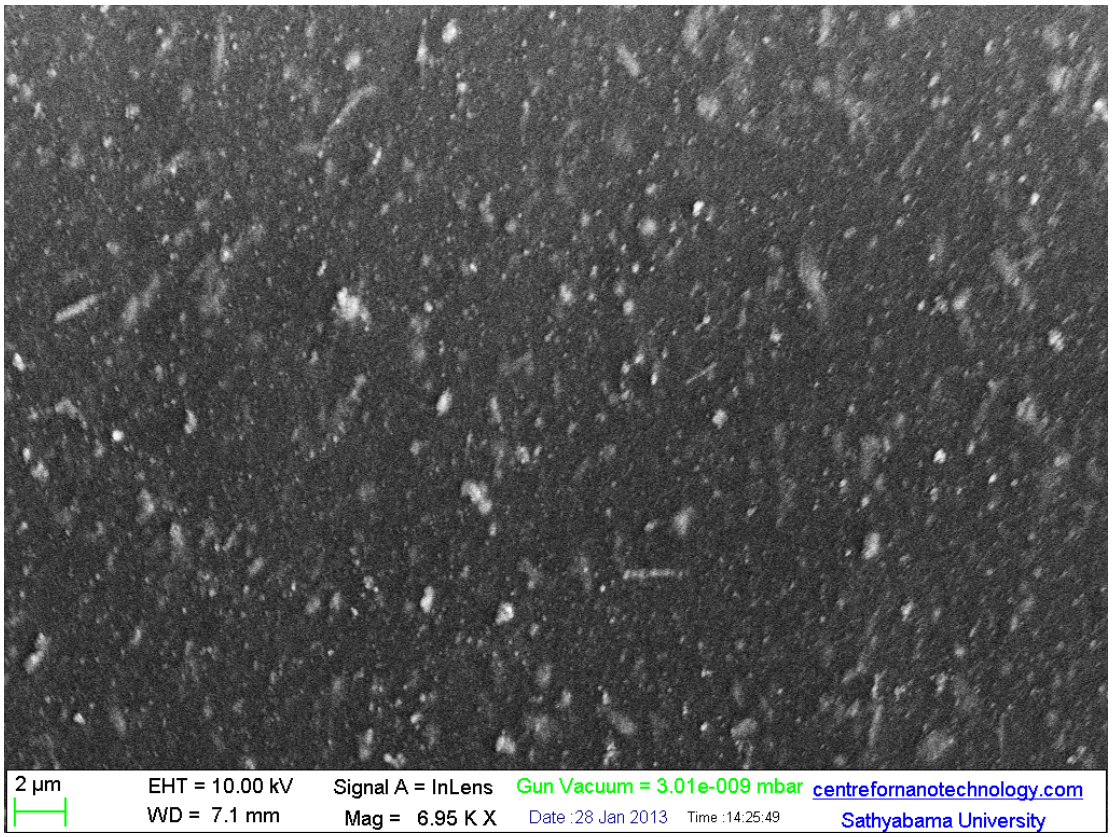


Table 1 : Biofilm inhibition (%) of *Staphylococcus aureus* by nanoparticles

S.No	Concentration (µg)	Biofilm inhibition (%)
1	10	42.1
2	25	59.4
3	50	69.0
4	75	75.5
5	100	84.0

Table 2 : Effect of nanoparticles on biochemical composition of biofilm matrix of *Staphylococcus aureus*

S.No	Concentration	Total Protein (µg)	Total carbohydrate (µg)
1	10	79.0	70.0
2	25	45.0	57.0
3	50	30.0	31.9
4	75	22.0	14.5
5	100	17.5	8.5
6	Catheter	13.0	5.0

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