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EVALUATING THE EFFECTIVENESS OF SILVER AND IRON NANOPARTICLES IN REDUCING BIOFILM FORMATION IN URINARY CATHETERS

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ABSTRACT

The present study made an attempt to use nanoparticles coated catheters for reducing the prevalence of biofilm in catheterized infections. Silverand ironnanoparticleswere synthesized using Sol-gelmethod.The nanoparticles were initially characterized by UV-Visible spectrophotometer and FTIR. Biofilm inhibition by silver and iron nanoparticle coated catheters was studied using biofilm inhibition, biofilm composition and bacterial adherence study.The biofilm inhibition was high in iron nanoparticles than silver nanoparticles.On analysing the biofilm matrix composition of protein and carbohydrate, increased composition were recorded in uncoated catheters and decreased level in iron nanoparticles coated catheters. FTIR spectrum of iron nanoparticles coated catheter showed more peaks.The additional peaks support the presence of compounds formed during nanoparticle synthesis. Iron nanoparticles were further characterised by SEM and EDAX. SEM image reported the presence of nanoparticles with some aggregates.The iron nanoparticles were found to be spherical inshape with approximately 150nm in size.EDAX analysis of Iron nanoparticles showed the presence of the element iron without any impurity.

Keywords: Biofilm, Nanoparticles, Catheters.

INTRODUCTION

Biofilms are universal, complex, interdependent communities of surface associated microorganisms. They are complex aggregations of microorganisms that can form irreversible attachments to the surfaces of living and non-living surfaces. The organisms are enclosed in an exopolysaccharide matrix occurring on any surface, particularly aquatic and industrial water systems as well as medical devices. (Donlan and Costerton 2002). Biofilm, related microbial infection in catheters 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. Bacterial infection from medical devices is a major problem and accounts for an increasing number of deaths as well as high medical costs. Many different strategies have been developed to decrease the incidence of medical device related infection. One way to prevent infection is by modifying the surface of the devices in such a way that no bacterial adhesion can occur (Knetsch and Koole, 2011).

Biofilms create an environment that enhances antimicrobial resistance. Colonisation of clinically implanted surfaces such as prosthetic hip implants, central venous catheters and urinary catheters can occur by biofilm forming bacteria(Hetrick and Schoenfisch, 2006).The risk of acquiring a Urinary tract infection (UTI) depends on the method of catheterization, duration of catheter use, the quality of catheter care, and host susceptibility (Crouzet et al., 2007).

The Present study is planned with an objective to study the effectiveness of iron and silver nanoparticles coatings in reducing the prevalence of Catheter associated UTI. The efficiency of nanoparticle coating is assessed through biofilm inhibition, biofilm composition and bacterial adherence study.

MATERIALS AND METHODS

Synthesis of Silver Nanoparticles

Silver nanoparticles were synthesized by chemical reduction of 0.1 M silver nitrate with 0.1 M Tri sodium citrate with 0.1 M sodium borohydride as reducing agent. Synthesis of silver nanoparticles was confirmed by the conversion of the reaction mixture into brown colour (Namasivayam et al., 2012).

Synthesis of Iron Nanoparticles

Ferrous sulphate and sodium borohydride was used to synthesize iron nanoparticles. 5g of ferrous sulphate was taken in a beaker and dissolved in 30ml methanol and 70ml of deionized water, pH was adjusted to 6.8. 2g

of sodium borohydride was dissolved in 10ml of deionized water. Sodium borohydride solution was slowly added drop by drop to the ferrous sulphate solution. The resulting mixture was then centrifuged at 5000rpm for 15minutes. Solid particle obtained was twice washed with methanol and dried in vacuum (Tang *et al*., 2010).

Characterization of Nanoparticles

Uv-Vis Spectral Analysis

Synthesized nanoparticles were further confirmed by sampling the aqueous component and the absorption maxima was scanned by UV-Vis spectrophotometer at the wavelength of 325 – 825 nm on Spectrophotometer (Anarkali et al., 2012).

Fourier-Infrared Spectroscopy Analysis

Nanoparticles were observed for infrared spectroscopy (FT-IR). The vibrational frequencies of chemical groups present in the samples were recorded (Kumar et al., 2012).

Scanning Electron Microscope and EDX

The structure and size of the silver nanoparticles was characterized by using Scanning Electron microscopy (SEM). The Energy dispersive X-ray analysis (EDAX) spectra also measured (Rajeshkumar et al., 2013).

Coating of Silver Nanoparticles on Catheter

The sterile catheter was cut approximately for 2cm and the cut pieces of the catheter were completely immersed in the respective mono dispersive colloidal nanoparticle suspension and kept at 37ºC for 48 hours. The coated catheters were placed on blotting paper to remove excess suspension and allowed to dry at room temperature. The coated catheter is used for further biofilm inhibition studies (Namasivayam et al., 2012).

Biofilm Inhibition by Plating Method

The coated catheter was incubated on the normal urine sample for 48hours.The catheters were plated in the MacConkey agar plates at different time intervals (0,6, 24,30,48hours). The bacterial growth was observed and recorded.

BIOFILM INHIBITION ASSAY

The silver nanoparticles coated catheter pieces were immersed in 10ml of 24hrs bacterial culture, incubated at 37ºC for 24hrs.After incubation period the treated catheter was stained with 0.1%weight by volume of crystal violet solution for 30minutes at room temperature, after staining the catheter was washed with 95% of ethanol for 3 times at room temperature,the washed solution was collected and read spectrophotometrically at 570nm. The percentage of biofilm inhibition

was calculated by following formula (Namasivayam et al., 2012).

%of inhibition = OD in control –OD in treatment *100 /OD in control

Evaluation Of Biofilm Composition

Coated catheters were cut into 2cm and immersed the urine sample for 48 hours. Catheter was removed and transferred to screw cap bottles containing 3ml of 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 and carbohydrates (Namasivayam et al., 2014).

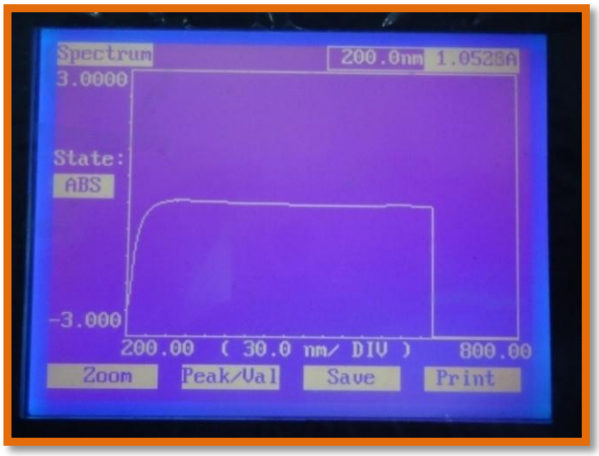
BACTERIAL ADHESION ASSAY

The inoculum was prepared by diluting overnight growth *S.epidermidis* and *P.aeruginosa* cultures to 1:10 in fresh BHI broth and incubated at 37°C for 2 hours with shaking at 100rpm. Catheter segment were immersed in urine for 30minutes. After 30min, the catheters were removed and placed in 15ml sterile culture tubes, each containing 10ml BHI broth. The tubes were inoculated with 100µl of bacterial inoculum (approximately $1x10^7$cells/ml) and incubated in a water bath for 3hours at 37^0C with shaking at 100rpm. After incubation, the catheter segment were washed three times in saline and transferred into a sterile 2-ml tube containing 1ml saline. Adherent bacteria were removed by sonication for 30s, followed by vortexing for 1min. The cells were serially diluted in saline and then plated onto LB agar plates. The plates were incubated at 37^0C for 24h, and colonies were counted **(**Burton*etal*., 2006).

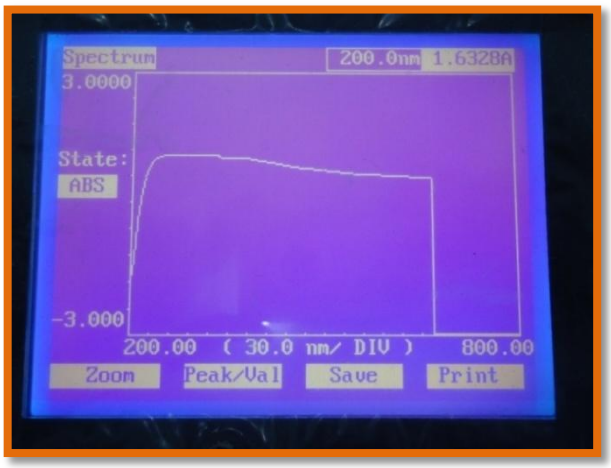
RESULTS AND DISCUSSION

Silverand iron nanoparticles were synthesized for studying their effect in bio film inhibition. UV absorption spectrum was measured for the synthesized nanoparticles.

The colloidal silver nanoparticles (AgNP) showed interrupted absorbance



peaks at 300-320 nm and reported in Fig 1.Iron nanoparticles (FeNP) showed



absorption maxima at 230- 350 nm and reported in Fig 2**.**

Fig 1 UV-Visible spectrum of silver nanoparticles

Fig 2 UV-Visible spectrum of iron nanoparticles

Change in colour was due to excitation of surface plasmon resonance (SPR) which is characterized by UV-VIS spectroscopy indicating formation Fe NPs (Song and Kim, 2009).The UV visible spectroscopy of the synthesized nanoparticles from curry leaves extract were in the range of 216-265 nm (Pattanayak and Nayak, 2013).

Fourier transform infrared spectrum of the iron nanoparticles and silver coated catheterswere reported in Fig 3 & 4. The absorption peak obtained at 742.54 cm^{-1} was assigned to the functional group C-H bend (o-disubstituted);

Fig3 FTIR analysis for iron nanoparticles

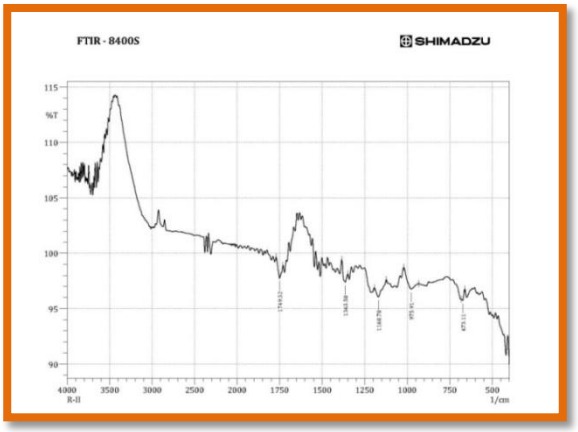


Fig 4 FTIR analysis for silver nanoparticles

A peak at 1074.28 cm^{-1} was assigned to the functional group C-F stretch (Primary alcohol); absorption peak at 1124.42 cm^{-1} was assigned to the functional group C-O stretch (Secondary alcohol); peaks obtained at 1461.94 cm^{-1} was assigned to the functional group C-H bend (Alkyls); peak obtained at 2960.53 cm^{-1} corresponds to the functional group C-H stretch (Alkanes)

The nanoparticle samples were analyzed in FTIR to identify the possible bio molecules responsible for the reduction of the Ag+ ions by the cell filtrate. The representative spectra of nanoparticles obtained manifests absorption peaks located at about 3843.68 cm-1 (–NH group of amines), 3597.73 cm-1 (-OH group of phenols), 2080.65 cm-1 (aromatic –CH stretching), 1631.66 cm-1 (-NHCO of amide) and 767.16 cm-1 (C-Cl) (Naveen et al., 2010). A new sterile catheter was cut approximately at 2cm and coated with the synthesized nanoparticles for 48 hours. Change in the colour of the catheter is indicative for the coating of nanoparticles .The coated catheter was incubated in the normal urine sample for 48 hours at room temperature. The catheter was plated in MaCconkey agar plate for assessing the presence of microbes on the coated surface at different time intervals (0, 5, 24, 31, 48 hours)and assessed thepresence of microbes

on the coated surfacein MaConkey agar platewhich is reported in Table 1.Silver nanoparticles were coated by single dispersion method on the catheter for 24 hours duration (Namasivvayam et al., 2012). Coating of biogenic silver nanoparticle was easily identified by color change of catheter (Namasivayam et al., 2013).

Table 1 Nanoparticles coating with different time intervals

Sample TIME	UCC	AgNP	FeNP
Coated (dry)	-	-	-
0hours (with urine sample)	+	-	-
6hours (with urine sample)	+	+	+
24hours (with urine sample)	+++	++	++
31hours (with urine sample)	+++	++	++
48hours (with urine sample)	++++	+++	+++

- No growth,
+ Minimum growth,
++ Moderate growth
+++ Maximum growth,
++++ Abundance growth

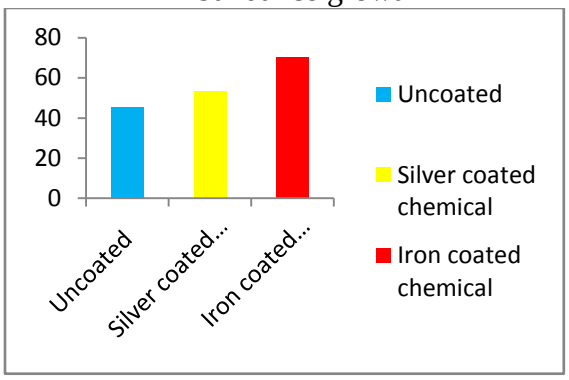


Fig 5 Biofilm inhibition assay

Biofilm inhibition was low (38.7% mg/catheter) in silver nanoparticle coated catheter compared to that of iron nanoparticle coated catheters (61.0- 75% mg/catheter)(Fig 5). The oPDM- plus-PS coating was more effective than silver hydrogel coating in inhibiting the adherence of *P.aeruginosa* and *S.epidermidis* to catheters. Furthermore, this combination was superior to nitrofurazone coating in inhibition *P.aeruginosa* adherence to catheters (Burton et al., 2006).

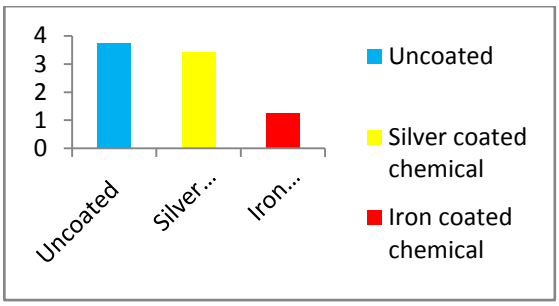


Fig 6 biofilm matrix – carbohydrate composition

Carbohydrate composition was high (3.72 mg/catheter) in uncoated catheter and very low level (1.26 mg/catheter) in iron nanoparticle (FeNp) coated catheter (Fig 6).

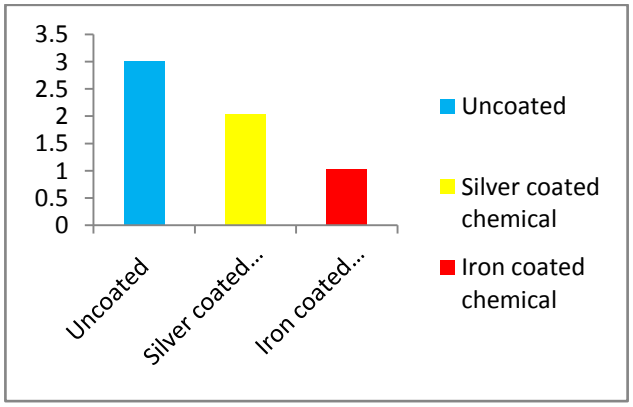


Fig 7 Biofilm matrix - protein composition

Protein in biofilm matrix was reported in Fig 7. Protein composition was high (3 mg/catheter) in uncoated catheter.Catheter coated with Silver nanoparticles (AgNP) showed thenext level (2.04 mg/catheter). Biochemical composition (carbohydrate and protein)of biofilm matrix in nanoparticle coated catheters were highly reduced.

The total carbohydrate reduced from 75.0, 55.0, 11.5, and 7.5 µg/mg 0.21, 0.36, 0.43, and 0.52 µg/mg in nanoparticles coated catheter with oflaxacin, cephalexin, neoflaxin and nanoparticles without antibiotics respectively (Namasivayam et al., 2012).

Table 2 Bacterial adhesion assay

Dilution Catheter	**10^{-6}**	**10^{-5}**	**10^{-4}**	**10^{-3}**	**10^{-2}**	**10^{-1}**
UCC	92	52	40	36	25	13
AgNp	30	18	14	8	6	4
FeNp	-	-	-	-	-	-

Bacterial adhesion assay (Table 2) was reported with no growth in FeNp followed by minimum growth in AgNp and maximum growth observed in uncoated catheters, thus supporting the potent efficiency of iron nanoparticle in controlling the biofilm formation.

Based on the previous application studies FeNp is selected for analysing by SEM and EDAX. SEM image was reported in Fig 8. The image showed the distribution of nanoparticles in the catheter. The size of the nanoparticle was found approximately as 150nm. EDAX analysis reported in Fig 9 confirms the presence of iron in the nanoparticles. Atomic weight of iron is found to be 41%.

The SEM analysis illustrates that the particles morphology was oriented spindle like with small spherical structure (Bagheri et al., 2013). In the EDX spectrum of the bacterial mediated silver nanoparticles, the strongest peak detected was from silver with weaker peaks from carbon and oxygen This indicates that the biological synthesis of silver nanoparticles is relatively unadulterated in chemical composition (Malarkodi et al., 2013).

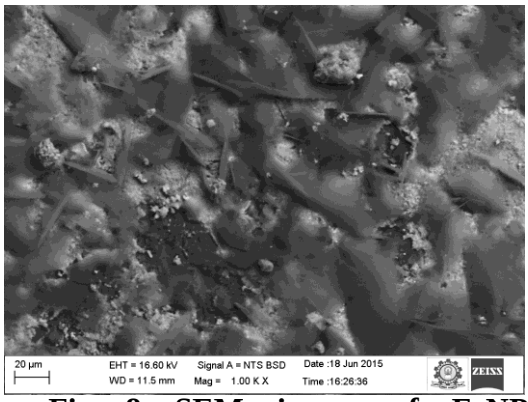


Fig 8 SEM image of FeNP

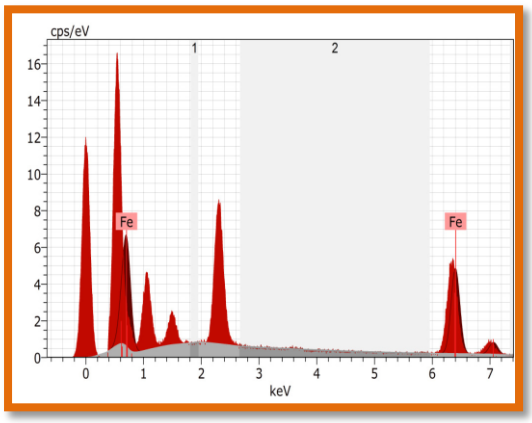


Fig-9 EDX analysis of FeNp

CONCLUSION

Based on the results obtained, it can be concluded that, iron nanoparticle coating in catheters can reduce biofilm formation in catheters. Thus iron nanoparticle coated catheters can reduce the prevalence of hospital acquired catheter associated urinary tract infections.

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