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COMPARATIVE STUDY ON ANTIBIOFILM ACTIVITY OF CAPPED SILVER NANOPARTICLES

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Abstract: The antibiofilm activity of silver nanoparticles synthesized via biological methods and chemical methods were investigated. Biofilms confer protection from adverse environmental conditions and can be reservoirs for pathogenic organisms and sources of disease outbreaks, especially in medical devices. Biological synthesis of silver nanoparticles using *Allium sativum* (As-AgNPs) and *Allium cepa* (Ac-AgNPs) and chemical synthesis of silver nanoparticles (Cs-AgNPs) using sodium citrate was carried out. These nanoparticles were used to evaluate the antibiofilm activities against several microorganisms of clinical interest. The results showed that both biologically synthesized (As-AgNPs & Ac-AgNPs) as well as chemically synthesized silver nanoparticles (Cs-AgNPs) prevent the formation of biofilms. Those silver nanoparticles synthesized using *Allium cepa* showed an enhanced antibiofilm activity as compared to that of As-AgNPs and Cs-AgNPs are lethal to bacteria in established biofilms, which suggests that these AgNPs could be effectively used for prevention and treatment of biofilm-related infections.

Key words: Bioreduction, Biosynthesis, Bacteria, *Allium sativum, Allium cepa*

INTRODUCTION

Biofilm, the aggregation of microorganism on a solid surface are responsible for many relentless and chronic bacterial infections, ocular related infectious diseases in human namely microbial keratitis. Biofilm mode of bacterial growth is one of the adaptive processes that allow bacteria to survive in nutrient-depleted environment. The biofilm bacteria show higher antimicrobial resistance as compared with other bacteria. The resistance of biofilm bacteria against antibacterial agents has been attributed to the protective barrier provided by the extracellular polymeric matrix (EPM) (Dunne *et al.,* 1983). Therefore, agents that possess the ability to disrupt the EPM would allow better penetration of antibacterial agents into the biofilm structure and result in significant bacterial elimination. Issues related to the effective elimination of bacterial biofilm and disruption of biofilm structure still remains in endodontic disinfection. Different approaches have been used for preventing biofilm related infections in health care settings. Various nanoparticles have gained popularity as antimicrobial agents as a result of their broad spectrum of activity and biocompatibility. Nanoparticles exhibit higher antibacterial activity as a result of their higher surface area and charge density, resulting in greater degree of interaction with the bacterial cell. It has been observed that the size of nanoparticles plays an important role in their antibacterial activity, with smaller particles showing higher antibacterial activity than the macro scaled ones. Metallic nanoparticles such as silver and gold nano particles are known to possess significant antibacterial properties. *Escherichia coli*, *Bacillus subtilis* and *Proteus vulgaris* has the ability to form biofilms, which renders drug-resistance and contribute to the pathogenesis of infection. This study aimed to test the efficacy of silver nanoparticles (AgNPs) in disrupting biofilm bacteria. We also checked the comparative antibiofilm activity of bio-synthesized and chemically synthesized silver nanoparticles.

MATERIALS AND METHODS

Synthesis of Silver nanoparticles

Silver nanoparticles were biologically synthesized using aqueous extracts of *Allium sativum* (As-AgNPs) and *Allium cepa* (Ac-AgNPs), which were used as reducing as well as capping agents, while in chemical synthesis

sodium citrate was used as capping and reducing agent. Silver nitrate was used as the precursor. Silver nanoparticles formed were characterized using different techniques such as UV-Visible spectroscopy, XRD etc.

The antibiofilm activities of silver nanoparticles were tested by antibiofilm assay. The overnight grown cultures were used for the study. Different concentrations of AgNPs were tested for its antibiofilm activity at different time intervals. For quantification of activity the crystal violet was used as the indicator and the absorbance was measured at 570 nm.

RESULTS AND DISCUSSION

In our study, the bio-reduction of aqueous Ag^+ ions by garlic and onion extract has been demonstrated. The reduction of the metal ions through these aqueous extracts leads to the formation of silver nanoparticles of fairly well-defined dimensions. Silver nanoparticles were formed by the reduction of Ag^+ from silver nitrate to Ag (o) atoms using extracts of *A.sativum* and *Allium cepa.* It is found that the Ag (o) atoms nucleate and form silver nanoparticles. While in chemical method sodium citrate was used as reducing agent to reduce Ag^+ to Ag^o and it also act as capping agent to control particle growth and prevent aggregation. Biofilm formation is detected in many organisms synthesizing exopoly saccharide. The biofilm is made up of microorganisms adhering to the surface coated with slime – the exopolysaccharide matrix which protects the microbes from the unfavorable environmental factors (Christensen, 1985). Among the bacteria tested for antibiofilm efficacy *Escherichia coli*, *Bacillus subtilis* and *Proteus vulgaris* have been studied in great detail with respect to their ability to form biofilm. In this study, we tested the effect of biosynthesized and chemically synthesized AgNPs (Cs-AgNPs) on biofilm formation under *in vitro* conditions by monitoring the binding of the crystal violet to adherent cells, which directly reflects the effective ability of the biofilm formation. *Bacillus subtilis, Escherichia coli* and *Proteus vulgaris* were grown in microtitre wells and were treated with varying concentrations of Ac-AgNPs, As-AgNPs, and Cs-AgNPs. In the case of *Bacillus subtilis*, As-AgNPs and Cs-AgNPs, treatment for 24 hours resulted in a decrease of more than 50% of the biofilm formation at 60 µg/ml, 80 µg/ml and 100 µg/ml concentrations, respectively (Fig. 1). While Ac-AgNPs shows a decrease of more than 80% at 100 µg/ml, whereas 48 hours treatment resulted in a decrease of more than 50 % in 100 µg/ml in Cs-AgNPs and As-AgNPs and in the case of Ac-AgNPs there is more than 90% decrease in biofilm formation at 100 µg/ml. These data indicate that Ac-AgNPs more effectively impede biofilm formation of *Bacillus subtilis*. In *E.coli* Ac-AgNPs shows more than 50% of antibiofilm property for both 24 and 48 hours (Fig. 2). While in *Proteus vulgaris*, the 24 hour treatment and 48 hour treatment with Ac-AgNPs shows a decrease of more than 75 and 88% respectively in biofilm formation at 100 ìg/ml when compared to that of As- AgNPs and Cs-AgNPs (Fig. 3).

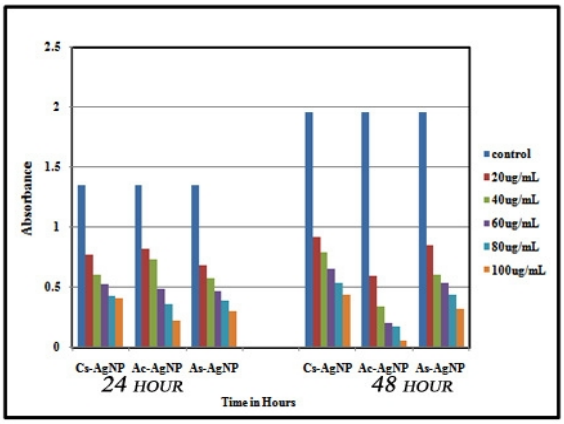

Fig .1. Antibiofilm activity of AgNPs at ifferent concentrations on *Bacillus subtilis*

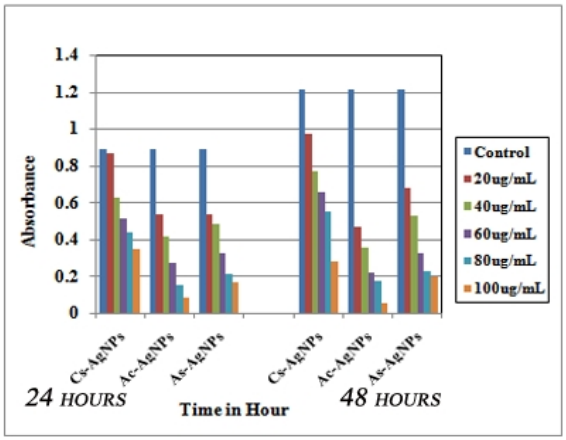

Fig.2. Antibiofilm activity of AgNPs at different concentrations on *Escherichia coli*

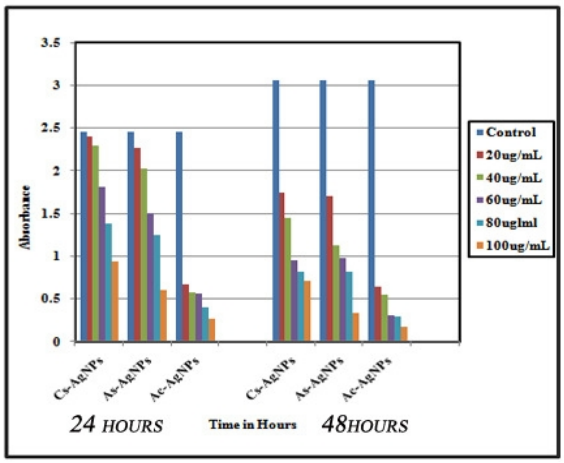

Fig.3. Antibiofilm activity of AgNPs at different concentrations on *Proteus vulgaris*

The recurrence of biofilms in spite of treatment with various anti-microbial agents was attributed to the impedance created by the biofilm matrix (Mohammed and Al-Fattani, 2006).Therefore, inhibition of biofilm formation is very much essential in the case of prevention of various disorders, as the exopolysaccharide slime formed reduces the susceptibility of the organism to the administered drug. Antibiotic treatments effectively kill the bacteria which remain individual, but the efficiency is very much reduced when the organism forms slime. This makes the organisms to revert the disease when the antibiotic treatment is finished because of the healing of symptoms. This is the reason behind the biofilm infections showing recurring symptoms even after cycles of antibiotic therapy until the mass is surgically removed. Therefore in this report we investigated whether AgNPs capped by different methods has antibiofilm function and the data indicate that Ac-AgNPs more effectively impede biofilm formation of *Bacillus subtilis*.

CONCLUSIONS

The ability to reduce the biofilm formation by silver nanoparticles can pave a way in controlling pathogenic organisms which have high efficacy of biofilm formation in the ecosystem and have great possibilities to be used as an effective antibiofilm agent to combat resistance problem. The increase in AgNPs concentration negatively regulated the biofilm formation. Biologically synthesized silver nanoparticles showed enhanced antibiofilm activity as compared to that of chemically synthesized silver nanoparticles (AgNPs). Among biologically synthesized silver nanoparticle Ac-AgNPs shows increased antibiofilm activity than that of As-AgNPs. The antibiofilm activity could be due to inhibition of exopolysaccharide synthesis because it has been shown that AgNPs impair exopolysaccharide synthesis and secretion. Applications of silver nanoparticles based on these findings may lead to valuable discoveries in various areas such as biomedical fields and can be used for prevention and treatment of biofilm-related infections

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