Original Article

Antibacterial effects of nickel nano-particles on biofilm production amounts by *B.capacia ATCC 25416*

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Abstract

***Background & Aims*:** B.cepacia is one of the causative agents of health care associated infections which have the ability of attachment to different surfaces and biofilm formation is one of the most important virulence factors in pathogenesis of this microorganism. Nanoparticles are key components which are considered for the designing of new antimicrobial agents, no studies have been done on the anti-biofilm effects of Ni-NPs on B.cepacia, so the aim of this study was to evaluate the anti-biofilm effects of different concentrations of Ni-NPs on B.cepacia.

***Materials & Methods*:** Microtiter plate method was used to determine the potential of the B.capacia ATCC 25416 in respect of biofilm production. The amounts of biofilm formation were also measured in the presence of 0.01, 0.1, 0.5 and 1 mg/mL concentrations of Ni-NPs. Statistical analysis was done by one-way ANOVA to determine significant differences between groups.

***Results*:** The study results revealed that B.capacia ATCC 25416 was strong biofilm producer. Biofilm formation significantly decreased in the presence of 1, 0.5 and 0.1 mg/mL of Ni-NPs (p=0.00, 0.00 and 0.008 respectively). Although in the presence of 0.01mg/mL of Ni-NPs decrease in biofilm formation was observed, but it was not statistically significant (P=0.08).

***Conclusion*:** The present study showed the ability of biofilm formation by B.capacia ATCC 25416. On the other hand, the lowering effects of nickel nanoparticles on biofilm formation by this microorganism were observed.

***Keywords*:** Burkholderia cepacia, nickel nanoparticles, biofilim

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Introduction

Members of Burkholderia Cepacia complex are negative gram bacteria, spore free, aerobic, vagile with respiratory metabolism and usually catalase and positive oxidase. They have been separated from different types of water resources and the environment in hospitals where they can be passed through to patients. The bacteria are the problematic respiratory pathogens in patients with cystic fibrosis. The small percentage of patients with cystic fibrosis are infected with Burkholderia Cepacia. However, since the infection could be serious, such as necrotizing pneumonia or bacteremia, the main risk is considered in these patients. Diagnosis of Burkholderia Cepacia infection in patients

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with cystic fibrosis is very important. Most bacteria are belonging to cepacia complexes that are separated from the hospital environment and people with cystic fibrosis and are multidrug resistance (1-4).

The biofilms of the bacteria include the congregation of bacterial microorganisms and extracellular protein products and polysaccharides available in the space between them attached to an inanimate or animate surface. Due to the special structure and the presence of extracellular polymeric material, biofilms reduce the physical and chemical antimicrobial effects and reduces the influence of anti-microbial agents including antibiotics. Biofilms do not disappear simply as a result of chemical and physical influences such as temperature controllers, drought, cleaners and detergents, as a result, the probability of survival of pathogenic bacteria in biofilms increases. Treatment of infections caused by biofilm production bacteria is difficult and physicians face so many problems in this area. Due to the importance of biofilm in diseases and drug resistance caused by it, the researchers have been seeking ways to control and prevent the formation of biofilms (5-7).

Metallic nanoparticles have been used for centuries by human as unknown. Nanoparticles are the groups of atoms, ions or molecules with a typical diameter of 1-100 Nanometer. This small size is valuable as nanoparticles, due to the small size, are able to penetrate the gaps of small molecules and create failure in the energetic condition. Nickel nanoparticles antimicrobial effects have been identified in limited studies on some bacterial strains such as Staphylococcus aureus and E. coli. The anti-biofilm effects on the clinical strains of Staphylococcus epidermidis and Staphylococcus aureus have been shown (8-11). Nickel nanoparticles are applicable in the treatment of diseases such as cancers and infections and enjoyed a variety of biological activities including anti-tumor activities. The components show Friendliness gap of DNA. These compounds form non-covalent DNA-bindings and react with H_2O_2 and create active compounds which can lead to cutting DNA strands, thereby induce cell death. One of the main limitations in successful eradication of Burkholderia Cepacia complex is its ability in biofilm formation (12, 13), designing and introduction of new and effective anti-biofilm is necessary for the treatment of such infections. Based on the studies in the literature, no study has been carried out about the effects of nickel nanoparticles on biofilms of Burkholderia Cepacia. The aim of this study was to investigate the effect of different concentrations of nickel nanoparticles in preventing biofilm formation and or its reduction by standard strains of Burkholderia Cepacia in order to introduce a new antimicrobial agent for prevention and control of infections as a result of biofilm formation by the bacteria.

Materials and Methods

Methodology:

This study is an interventional which used different concentrations of nickel nanoparticles in the form of nano-powder to highlight the changes in the formation of biofilm formation or inhibition of its production by standard strains of Burkholderia Cepacia.

Preparation of standard strains of Burkholderia Cepacia:

Burkholderia Cepacia standard strain of ATCC 25416 was bought from microbial collection of biological resources of Iranian Biological Research Center as Lyophilized injections and was confirmed after its restoration in terms of microscopic form, germ response, colony morphology and biochemical characteristics and then entered the study. The effects of different concentrations of nano-nickel powder on a strain of keeping have been evaluated.

Preparation of nickel nanoparticles:

Nano-powder Nickel nanoparticles purchased from Sigma - Aldrich have been suspended in TSB medium

and was used for subsequent experiments. The sonicated were used for two hours before the experiment to dissolve the particles. The average size of the nickel nanoparticles according to the purchase catalog was less than 100 nm.

Study of biofilm production:

To determine the presence and amount of biofilm produced by standard strain, bacterial strains were grown overnight in TSB medium. The culture was then diluted to about one hundredth and 100 microliters of the diluted to the contents of the medium was added to each 96 –part microplate covered wells and was incubated at 37 ° C for 24 hours. After incubation, the plate contents were drained and immersed in microplate water bath. The water is then discharged by shaking microplate wells and washed twice with water. In order to stain the biofilm, 125 ml of crystal violet solution %0.1 in water was added to each well and incubated at room temperature for 10-15 minutes. Then the microplate 3-4 Bar was risen out through immersion in bath water and then dehydrated and dried. 125 ml of 30% acetic acid in water was added to each well to solve violet crystal and incubation was carried out at room temperature for 10-15 minutes. 125 ml of the contents of each well was transferred to a new flat bottom micro titer plate and the absorbance was read in 550 nm using solution 30% of acetic acid as blank. To ensure the resulting, all experiments were repeated at nine wells in accordance with existing guidelines (14). Comparing of absorption rates with negative control containing only TSB medium was used to determine the ability to produce biofilm by standard strains of Burkholderia Cepacia.

For grouping biofilm under study in terms of productivity, the amount of absorption compared with the negative control samples was used according to the following table (8, 15).

Table 1: Classification of bacteria in terms of the biofilm production power

Lack of biofilm formation	ODs ≤ODc
Poor production of biofilm	ODc≤ ODs ≤ 2×ODc
Average production of biofilm	2 × ODc ≤ ODs ≤ 4 × ODc
Strong production of biofilm	4 × ODc <ODs

ODc = density of the negative control ODs = density of the bacterial samples

Study of bacterial biofilm formation in the presence of nickel nanoparticles:

In order to measure the effects of nickel nanoparticles on biofilm production by itself, the above method was repeated, except that each nine wells have been added nickel nanoparticles at concentrations of a hundredth, tenth, half or one milligram per milliliter (according to sources in accordance with the concentrations used on mucosal surfaces including dental materials) and suspended in culture medium and the mean absorbance obtained for each biofilm production strains were compared in the presence and absence of nickel nanoparticles. it should be noted that based on previous studies in the laboratory, no concentrations were not bactericidal and not able to inhibit the growth and proliferation of the bacteria.

The mean and SD for absorbance values obtained for the amount of biofilm formation by strains under investigation in nine wells in the presence of each test concentrations of nickel nanoparticles have been measured using Excel program and compared with the absence of nanoparticle. One-way analysis of variance (ANOVA) was used to analyze the results and for comparison between groups and the mean differences had been considered as significant when $p < 0.05$. Exel software was used to draw diagrams.

Results

Biofilm formation ability:

The ability of biofilm formation in Burkholderia Cepacia strain of ATCC 25416 for nine repeat was measured by measuring optical density through ELISA-Reader at wavelength of 550 nm and it was concluded that Burkholderia Cepacia bacteria strongly produces biofilms.

The study of biofilm formation by bacteria in the presence of nickel nanoparticles

The inhibitory effect of nanoparticles of nickel on the biofilm formation was assessed in concentrations of 1,0.5, 0.1 and 0.01 mg per milliliter. Nine wells were considered separately for each concentration. Results compared with the production of biofilm in the absence of nano-nickel is presented in Table 2.

Table 2: reading results of biofilm production using Elisa reader with bacteria in the presence and absence of various nickel nanoparticles concentrations

The mean ± SD of biofilm formation in the presence / absence of various concentrations of nanonickel with 9 rep.	B.cepacia ATCC 25416	(Nano-Ni) (mg / ml) Ni-NPs			Negative control TSB medium
	(0 mg/mL)	1	0.5	0.1	0.01	
Mean± SD	0.173±0.08	0.024±0.022	0.047±0.053	0.08±0.04	0.10±0.05	0.032±0.006
Biofilm formation	++++	0	+	+	+	0

Statistical analysis of mean comparison between groups showed that the biofilm formation difference between first group (Burkholderia Cepacia bacteria biofilm formation in the absence of nano-nickel) and second group (biofilm formation in bacteria Burkholderia Cepacia in the presence of nickel nanoparticles at a concentration of 1 mg ml), third group (Burkholderia Cepacia bacteria biofilm formation in the presence of nickel nanoparticles in a concentration of 0.5 mg per mL) ($P = 0.000$), and forth group (biofilm formation of bacteria Burkholderia Cepacia in the presence of nickel nanoparticles at a concentration of 0.1 mg per mL) is significant ($p = 0.008$), but the difference of biofilm formation between group 1 and 5(biofilm formation in bacteria Burkholderia Cepacia in the presence of Nickel nanoparticles at concentration of 0.01 mg per mL) is not significant although close to significant ($p = 0.08$).

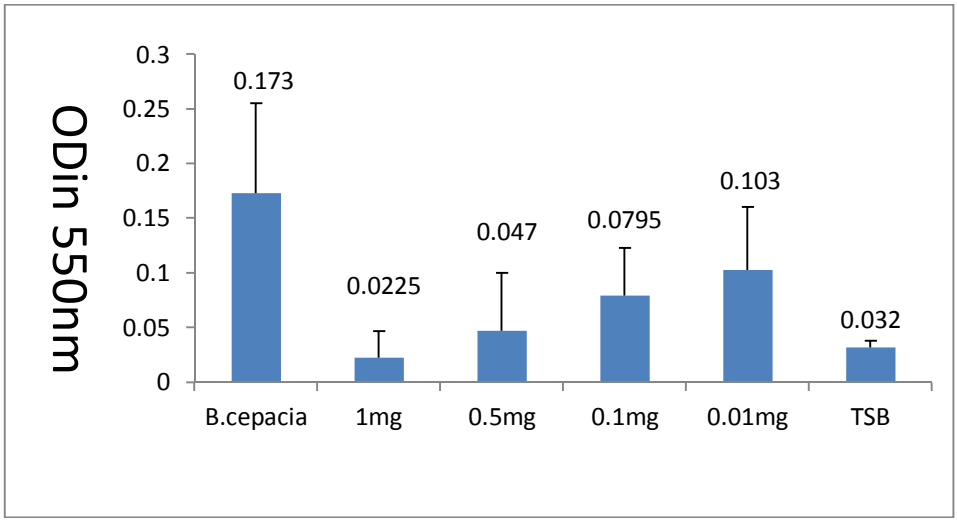

Figure 1: Biofilm producing bacteria in the presence and absence of nickel nanoparticles

1 (the ability to produce biofilm of Burkholderia Cepacia bacteria in the absence of nano-nickel)

2 (Ni nanoparticle effects at a concentration of 1 mg ml)

3 (Ni nanoparticle effects at a concentration of 0.5 mg per ml)

4 (Ni nanoparticle effects at a concentration of 0.1 mg per ml)

5 (Ni nanoparticle effects at concentrations 0.01 mg ml)

The ability to produce biofilm of Burkholderia Cepacia bacteria in the presence of 1 milligram per milliliter of nickel nanoparticles can be inhibited, but in concentrations of 0.5 and 0.1 milligram per milliliter, the biofilm formation in Burkholderia Cepacia bacteria is significantly decreases and in the concentration of 0.01 milligram per milliliter of nanoparticles of nickel, the formation of bacterial biofilm is also reduced, although this decrease is not significant.

Therefore, it can be concluded, among the four tested concentrations of the nickel nano particles (1, 0.5, 0.1 and 0.01) that their anti-biofilm effects have been studied, that the best inhibitory effect of nickel nanoparticles on the production of biofilm bacteria Burkholderia Cepacia is at a concentration of 1 mg per ml which completely inhibits the biofilm formation by the bacteria to

Discussion

Burkholderia Cepacia is one of bacteria causing nosocomial infections which has the ability to bind to and different levels and biofilm formation is one of the most important factors in the pathogenesis of this bacterium. Nanoparticles of silver and copper have antimicrobial properties that have been investigated in a few studies, but the effect of these particles on microbial mechanism is not fully understood.

Nickel is commonly used in the preparation of unpainted stainless steel and other metal alloys. Despite the extensive application of the metallic element in the alloy, properties of pure nickel are not known precisely. Alloys containing nickel are usually more resistant to heat and corrosion and are used in the chemical, food and medical industry (surgical instruments, orthopedic implants, syringe, etc.) (16-18).

Different types of nanoparticles such as copper, zinc, titanium, silver and nickel have antimicrobial properties against bacteria, viruses and microbial eukaryotes. Some of these compounds are able to inhibit biofilm formation by bacteria as well. In recent years, various methods have been proposed in order to inhibit biofilm

formation by bacteria. In recent years, nanoparticles have been considered as one of the most useful tools in containing and controlling biofilm formation by bacteria. Antibacterial properties of nano-Ni has been previously shown, but only a few studies have been carried out about the anti-biofilm effect of this compound on hospital Staphylococcus epidermidis strains and a standard strain of S. mutans and mupirocin-resistant strains of Staphylococcus aureus (19).

In 2015, Vahedi et al studied the antibacterial effect of nickel nanoparticles on the amount of biofilm formation in 22 isolates of Staphylococcus epidermidis. The biofilm formation in the presence of 0.01, 0.1,0.5 and 1 liter of nickel nanoparticles was measured. The results indicated that biofilm formation in the presence of 0.1, 0.5 and 1 mg ml of nickel nanoparticles was significantly decreased ($p < 0.05$), although a reduction in the amount of biofilm formation was observed in the presence of 0.05 mg per ml, this reduction was not significant (5). Thus, the above result is in agreement with the results in this study which shows a significant decrease in Burkholderia Cepacia biofilm formation in the presence of 0.1, 0.5 and 1 mg per ml of nickel nanoparticles (9).

In 2013, Mamonova et al investigated antibacterial metallic effects of nickel and titanium nanoparticles on clinical isolates of E. coli. The researchers noted that E. coli is the most common blood infection and other infections in human being. These bacteria use several mechanisms including ESBL enzymes production and carbapenemase for resistance to different antibiotics. Hence, the need to design or identify new antibacterial agents for the treatment of infections caused by these bacteria is necessary. Metal nanoparticles are potential candidates for to introduce new group of anti-bacterial agents. Metal nanoparticles toxicity is less in comparison with metal ions and have the ability of their administration in vivo and have long-term effects. In this study, antibacterial effects of nanoparticles of nickel and titanium on clinical isolates of E. coli were investigated and it was determined that nickel nanoparticles have efficient bactericidal activity. Antibacterial effects of nickel nanoparticle are concentration and exposure-time dependent. Therefore, the authors suggested that it is better to do further studies on antibacterial activity of nickel nanoparticles as an antibacterial agent (20).

In 2015, Mortazavi et al. studied the effect of colloidal silver nanoparticles on bacterial growth and biofilm formation of Staphylococcus epidermidis. In this experimental study, tests were performed on 13 clinical isolates and a standard strain of Staphylococcus epidermidis (ATCC 12228). Minimum Inhibitory Concentration (MIC) and MBC of nanoparticles were determined by agar dilution method. Investigation on biofilm formation was performed using a micro titer plate and staining with Safranin. The results showed the bacteriostatic effect of nanoparticles at low concentrations. The nanoparticles had anti-biofilm effect at the concentration of 0.5 ppm and the anti-biofilm effect of increased with an increase in concentration to 4 ppm. Also, nanoparticles at concentrations above 150 ppm was capable of destroying biofilm formation. The results showed that the silver colloidal nanoparticles are able to inhibit biofilm formation of Staphylococcus epidermidis. The researchers suggested that these compounds can be used in prosthesis coatings and prosthetic devices implanted in the body or covering surfaces in medical centers (21).

In 2015, Gahremani and colleagues studied the inhibitory properties of nanoparticles of nickel with a concentration of 10 mg per ml on biofilm formation by 30 isolates of Staphylococcus aureus resistant to mupirocin. Nickel nanoparticles inhibitory effect on biofilm formation of Staphylococcus aureus resistant to mupirocin in total in comparison to the amount of biofilm formation was significant compared with strains in the absence of nanoparticles ($p = 0.039$) (6). Although the concentrations used by the researchers are higher

than the concentrations tested in the present study, it is in line with present study that represents the anti-biofilm properties of nickel nanoparticles. The study revealed that the anti-biofilm effect of nickel nanoparticles was dose-dependent and the effect can increase with increasing concentration of nickel nanoparticles (8).

According to the findings of research, in case of the safety of the nanoparticles of nickel, effective concentrations of nanoparticles of nickel can be used in the prevention or elimination of biofilm produced by the bacterium Burkholderia Cepacia in hospital environments, or facilities in hospitals and in the case of complementary studies, can be used on laboratory animals to treat localized infections, or systemic applications. The limitations of this study are the evaluation of the anti-biofilm nickel nanoparticles on a single standard strains of Burkholderia Cepacia, it is suggested that future studies to be done on clinical isolates, especially variants isolated from patients with cystic fibrosis with a high and strong probability of biofilm production.

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