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Evaluation of Antibacterial Activity of Iron Oxide Nanoparticles against *Staphylococcus aureus*

Harini .S

MBBS, Saveetha Institute of Medical and Technical Science, Chennai 602105, India

Abstract: ***Biofilm is important virulence factor play an essential role in bacterial pathogenicity through trigger antimicrobial agents. Nanoparticles have antimicrobial properties and the antimicrobial activity depend on nanoparticles size and concentration. This study demonstrated the antimicrobial activity of iron oxide nanoparticle against Gram-positive bacteria within 20 hours. The particles obtained had an average diameter 25 nm; the particles were used to inhibit Staphylococcus aureus biofilm on polystyrene surface. The bacteria were added in 96-well plates to incubate with iron oxide nanoparticles and without iron oxide nanoparticles as control. The biofilm was measured using the safranin staining method that showed variable results depending on bacterial species. However, It was seen that exposure of cells to iron oxide particles showed increased biofilm formation on polystyrene plates. The highest augmentation was recorded for S. Aureus in a concentration of 0.5mg/ml and approaching (78.8%) highly significant p≤0.05 and the augmentation was not achieved in a concentration of 50 mg/ml p>0.05, The augmentation of S. Aureus in a concentration of 5mg/ml gave (75.8%). Our result indicated that iron oxide nanoparticles have not antibiofilm activity against bacteria cells within 20 hours for S aureus except at concentration 50mg/ml for S.aureus gave (18.2%) inhibition rate. The IONPs efficacy depends on the incubation time, bacterial strain and nanoparticles concentration.***

Keywords: *Staphylococcus aureus*, Iron oxide nanoparticles, Augmentation, Virulence factor and Biofilm.

1. Introduction

Iron oxide (IO) has been widely used in biomedical research because of its biocompatibility and magnetic properties. Nanoparticles of IO, with sizes less than 100 nm, have been developed as contrast agents for magnetic resonance imaging (MRI), as hyperthermia agents and as carriers for targeted drug delivery to treat several types of cancer. It is further believed that through the use of magnetic nanoparticles, an optimal drug delivery system can be developed by using an external magnetic field to direct such nanoparticles to desirable sites (such as implant infection) for immediate treatment. *Staphylococcus aureu*s is one of the most common human pathogens, and leads to many types of infection. This bacterium is responsible not only for local infections, such as wounds or postoperative infection, but also for prosthetic infection (such as through the use of catheters, endotracheal tubes, and other biomaterials).[1]

Staphylococcus aureus, a gram positive micro organism. Infection with staphylococci is almost always accompanied by accumulation of large amounts of pus. In fact, staphylococci are responsible for more than 80% of all suppurative infections and are capable of causing diverse conditions such as boils, folliculitis, pneumonia, acute enteritis, burn infections with bacteremia, scaled skin syndrome and toxic shock syndrome [2].

The antibiotic era, barely 60–70 years old, is also threatened because of, increase resistance rhythm of this organism against different antibiotics. In the past few years, the rates of morbidity and mortality are increasing due to MRSA infection, so studies in controlling these infections are gravely required. In addition to antibiotic resistance, the other factor that causes treatment failure and chronic and recurrent staphylococcal infections in burn patients is biofilm formation in these strains [3]. Since the ability of biofilm production by MRSA increases antibiotic resistance, hospital patients infected with these strains are at serious risk for treatment failure. Biofilm formation is considered to be a virulence factor because the microorganisms that establish in a burn wound biofilm fundamentally differ from suspended populations. Biofilm acts as a barrier to antimicrobial agents and the host immune system that assists sustained bacterial colonization. [4] Over the years, *S. aureus* has evolved and developed multiple strategies to evade human immune system and to resist antibiotics treatment. This has given rise to the evolution of MRSA, and the emergence of health care associated (HA) and community-associated (CA). MRSA has caused a major problem to the human society. MRSA are resistant to all β-lactam antibiotics, some to multiple antibiotic classes. Anxiety regarding the future availability of effective chemotherapeutic options resulted from resistance to all known antibiotic classes within the species, which in turn has occurred due to mutation and horizontal gene transfer. Since 1990s in many countries, prevalence of MRSA has increased dramatically [5].

S. aureus is also known to possess an increasing ability to resist antibiotics (such as penicillin, methicillin, tetracycline, erythromycin, and vancomycin). Thus, it is necessary to find an alternative treatment (perhaps without the use of antibiotics) for *S. aureus* infection that is directed to the site of infection, localized, and difficult for bacteria to formulate resistance. Along this line, some have hypothesized that reactive oxygen species (ROS) generated by Fe3O4 nanoparticles could kill bacteria without harming non-bacterial cells. [6]

The present study demonstrates that MRSA are strong biofilm producers and proceeding treatment with antibiotics made complications. Hence newer generative drugs like Iron oxide nanoparticles can be applied effectively for the control of microorganisms and the prevention of infections caused by MRSA. The exact mechanism is not clearly understood but the probable mechanisms include, role of reactive oxygen species (ROS) generated on the surface of particles,

ferric ion release membrane dysfunction, membrane internalisation, intracellular biotransformation of the nanoparticles or that the nanoparticles have dissolved to iron to enact toxicity. The antibacterial rate was 99.45% to *S. aureus.* Iron oxide nanoparticles were most effective for MRSA and it was suggested to have a strong affinity to the cells of MRSA.

2. Materials and Methods

2.1.1. Sample collection:

This Prospective study of 6 months duration was conducted at Microbiology laboratory in Saveetha medical college and hospital at Chennai.

Staphylococcus aureus was used for this study. Bacterial strains used in this study were obtained from the nosocomial infected patients. Bacteria were first grown aerobically overnight at 37°C in tryptone soy broth (TSB; Hi media, Mumbai, India) for 20 h.

2.1.2. Coagulase test to differentiate *Staphylococcus aureus* from other *Staphylococcus* species

The coagulase test detects the presence of free and bound enzyme which is called coagulase. This enzyme is excreted extracellularly by human strains of *Staphylococcus aureus*.

2.1.3. Materials

Iron Oxide NPs (product number from Sisco Research laboratories) of <25 nm particle size were used in this study. 180 µl of the iron-oxide nanoparticles were introduced in different concentrations (50 mg/ml , 5mg/ml and 0.5 mg/ml).

2.1.4. Confirmation of methicillin-resistant S. aureus (MRSA)

Bacterial suspension of all S. aureus isolates equivalent to 0.5 McFarland was prepared and cultured on Mueller-Hinton agar medium containing 2% NaCl and Cefoxitin antibiotics (6 µg/mL), then incubated for 24 hours at 30°C. The growth inhibition zones were interpreted using Clinical Laboratory Standards Institute (CLSI) guidelines

2.1.5. Effect of Iron-Oxide Nanoparticles on Biofilm Growth

In this study, bacterial adhesion on polystyrene well plate and bacterial with IONPs adhesion on polystyrene well plate were compared. 20 µl of *Staphylococcus aureus* and *Escherichia coli* culture suspensionwas added to each well. Then, 180 µl of the iron-oxide nanoparticles were introduced in different concentrations (50 mg/ml , 5mg/ml and 0.5 mg/ml). Thereafter, biofilms were allowed to grow for 20 h. Subsequently, wells were washed with sterile water to remove unbound bacteria then, 200 µL of 0.1% safranin staining was added to each well. Plates were incubated for 10 min. The wells were washed with sterile water and allowed to dry completely. Biofilm development was assessed by measuring the optical density (absorbance at 490 nm) using a BioTic ELISA reader (ELx800) (9). Experiments were performed in triplicate with separately cultured bacteria.

2.1.6. Statistical analysis

Experiments were performed in triplicate. Data are represented as a mean with standard deviation. For statistical analysis ANOVA was performed by a computer program for Epidemiological statistics and a p value <0.05 was considered to be significant.

3. Results and Discussion

Out of 200 skins and soft tissue infecion sample, 50 were determined as MRSA.

Coagulase test

The formation of a clot in the bottom of the tube is considered a positive result. The clot will not move as you tilt the tube. Un clotted plasma will flow in the tube.

Effect of Iron oxide nanoparticles on S.aureus biofilm formation

Iron oxide nanoparticle was used at concentration of 50, 5, 0.5 mg/ml on *S. aureus* biofilm in table (1), (2). The results indicated ainduction in the amount of biofilm biomass in the 20h treatment time compared with control.

Table 1: Effect of concentration of iron oxide nanoparticles on *S. Aureus* biofilm formation

Concentration mg/ml	*%Augmentation (Mean)*
50	0%
5	75.8%
0.5	78.8%

augmentation was calculated using the formula : % augmentation=Test O.D -Control O.D×100 ControlO.D

Table 2: Optical density (OD) biofilm of *S. aureus* with iron oxide nanoparticles on polystyrene surface

Concentration mg/ml	OD at 490nm (Mean + SD)	P-Value	T-test	Standard error
50	0.27±0.05	0.179	1.62	0.037
5	0.58 ±0.34	0.27	1.26	0.198
0.5	0.59 ±0.08	0.007	5.034	0.052
Control	0.33 ±0.04			

Increased biofilm formation were seen for Gram positive bacteria (table I, II). The highest augmentation significantly was seen at concentration 0.5mg/ml IONPs gave 78.8 % for *S.aureus.*, therefore IONPs used in bacteria biofilm building at low concentration through 20 hours this results was compatible with (7) who's mentioned that increased biofilm mass dependent on IONPs size .Iron used in biofilm regulation (8). In concentration of 50 mg/ml for *S.aureus* shown non significant decreased in biofilm formation and the inhibition gave 18.2%. No inhibition seen in *E.coli* but at concentration 50 mg/ml gave the lowest augmentation rate (36.9%) consequently increased IONPs concentration decreased biofilm formation (9). In addition *S.aureus* as gram positive cell wall different from *E.coli* gram negative cell wall. In gram negative bacteria, the cell wall is protected by outer membrane . While gram positive bacteria contain different types of peptidoglycan, vary in the amino acid (10), So we seen different results depending on the structure of the cell wall and the mechanisms to inter the IONPs.

Taylor and Webster showed that iron-oxide nanoparticles in a concentrationrange of 0.01 to 2mg/mL were inhibited 25% of *S.epidermidis biofilm* at 48 h (11). And, similar results were observed by (12) on *S. aureus*biofilms at 24 hours. In contrast, (13) showed an increase in *P. aeruginosa* biofilm biomass in the presence of 0.2mg within 16 hours. (14) results was similar with current studies with iron oxide nanoparticles on *S. aureus and E.coli* biofilms within 20 hours, consequently efficacy of IONPs to kill bacteria biofilm depends on the incubation time and nanoparticles concentration. So when the concentration of the nanoparticles increase the time is decrease to inhibited the biofilm and versa visa.

Agglutination are based concerning the availability over antibodies within patient sera that can react with specific antigens or structure visible clumps, but formation on biofilm may shield bacteria out of the assignment regarding antibodies (15).

The positive reaction between surface antigens about microorganism and the antibodies, as reflect on consideration on as like a good method ancient for diagnose infection then become awake of bacterial isolates with the aid of discovery on bacterial-specific antibodies of samples.

Study showed the polyclonal antibodies go commotion with hateful non-specific antigens, the awareness about antibodies who was once ancient toughness no longer ample because inhibition about the function about partial proteins (16). Specific antibodies blocked biofilm development at the initial attachment and aggregate stages, deletion and inhibited normal biofilm formation. So particular antibodies additionally respect namely opsonins after enhance neutrophil binding, motility, and biofilm engulfment.Vaccination against, or treatment by antibodies reactive to proteins may deliver targets for usage against a extensive spectrum of gram-positive bacteria.

Commonly *S. aureus* have an informed capacity to link non-specifically to bare polymer surface, this combination can be blocked by cover the surface with altered proteins, vaccine or as a goal in passive immunotherapy or prophylaxis. (17).

In this study, the concentration of nanoparticles was a major contribution to *S. aureus* activity inhibition. A similar concentration-dependent behavior was observed by Kohler et al when they investigated the antimicrobial effects of Ag and ZnO nanoparticles on *S. aureus* and *E. coli*.

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