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Magnetite nanoparticles influence the efficacy of antibiotics against biofilm embedded *Staphylococcus aureus* cells

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ABSTRACT

The expansion of bacterial antibiotic resistance is a growing problem today, and has determined the intensification of studies for finding out new alternatives to antibiotic treatment. The purpose of this study was to investigate the potential of magnetite nanoparticles (Fe_3O_4) to achieve a sustained and controlled drug release and subsequently improve the efficacy of antibiotics against *Staphylococcus aureus*, one of the most frequently isolated opportunistic pathogens, responsible for severe infections in immunocompromised patients with indwelling catheters or other biomedical devices. The obtained results showed that Fe_3O_4 nanoparticles functionalized with different antibiotics exhibited an inhibitory activity on growth and the biofilm formation of *S. aureus* strain superior to that exhibited by each antibiotic alone. The studied magnetic nanoparticles could act as efficient antibiotic potentiators and delivery systems for combating *S. aureus* biofilms on biomedical devices or human tissues.

Keywords: *magnetite nanoparticles, antibiotic resistance, medical device infection, Staphylococcus aureus, biofilms, minimal inhibitory concentration.*

1. INTRODUCTION

S. aureus is an extremely versatile human pathogen responsible for a broad range of nosocomial and community-acquired infections. Many of these infections involve biofilm formation, being very challenging due to resistance of bacteria from biofilm to both host immune responses and available chemotherapies [1,2]. Biofilm formation, especially on medical implants such as catheters, is an important virulence mechanism for methicillin-resistant *Staphylococcus aureus* (MRSA) contributing to the chronicity of infections. The emergence of MRSA strains as potentially lethal pathogens is a continuing cause for public health concern worldwide [3]. In Romania according to EARSS 2008 the proportion of MRSA was 33% [4]. The mortality rate due MRSA infections in a Romanian hospital from Bucharest was 52%, being registered during a study performed between July 2007 and June 2008, carried out in 13 tertiary care hospitals from European countries in order to estimate the excess mortality and length of hospital stay associated with MRSA bloodstream infections in European hospitals [5]. Therefore, it is extremely important to design and develop new alternatives to antibiotics for the treatment of biofilm infections with multi-drug resistant MRSA [6]. A new approach against biofilm-mediated, drug-resistant, and device centred infections is

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represented by the use of nanoparticles. Recently nanoparticles have been used successfully for the delivery of therapeutic agents [7] in chronic disease diagnostics [8], to reduce bacterial infections [9], and in the food and clothing industries as antimicrobial agents [10]. Magnetic iron oxide nanoparticles have raised much interest during the recent years due to their novel properties (superparamagnetism, high saturation field, blocking temperature, etc.) and potential applications in organic synthesis, biotechnology and in medicine, including: controlled drug delivery systems, magnetic resonance imaging, magnetic fluid hyperthermia, macromolecules and pathogens separation, cancer therapy [11]. Magnetic nanoparticles consisting of magnetite (Fe_3O_4) possess unique characteristics that make them promising as carriers in biomedical utilizations. Their properties, including thermal, chemical, and colloidal stability are not presented by other materials used for medical applications [11]. Despite the efficacy of nanocoatings to prevent biofilm formation on catheters, there is an important limitation, i.e. the ability of the material to adsorb always the same concentration of the drug and also the ability to control their release, which in most cases results in a non-controlled elution of the drug in the first hours subsequent to the insertion [12,13,14]. In this context, the objective of this study was to investigate the potential of magnetic nanoparticles to potentiate, but also to accomplish a sustained and controlled drug release and subsequently improve the efficacy of antibiotics against planktonic and biofilm growing *S. aureus* cells.

2. EXPERIMENTAL SECTION

2.1. Materials. All chemicals were used as received and were purchased from Sigma-Aldrich ChemieGmbh (Munich, Germany).

2.2. Fabrication of nanostructure. Functionalized magnetite nanoparticles (Fe_3O_4@ATB, ATB = penicillin (P), streptomycin (S), erythromycin (E), kanamycin (K) and cefotaxime (CTX)) were prepared and characterized according to our recently published papers [15,16].

2.3. Qualitative and quantitative assessment of the influence of Fe_3O_4@ATB on planktonic cells growth. The study of antimicrobial activity of Fe_3O_4@ATB against *S. aureus* ATCC 29213 reference strain was performed by a qualitative method for antimicrobial susceptibility testing based on disk diffusion following CLSI 2012 recommendations. In brief, the inoculum represented by a bacterial suspension from a 16-18 h culture developed on solid medium, and adjusted according to McFarland 0.5 standard was seeded on a Muller-Hinton Agar (MHA) medium plate. After inoculation plates were left standing for 10 minutes to let the culture get absorbed. Afterwards, the antibiotic disks, and antibiotic disks plus 10µL of Fe_3O_4@ATB, respectively were placed at corresponding distance on MHA plates, and plates were incubated la 35 ±2 °C. The results reading were performed by comparatively measuring of inhibition zone diameter generated by different antibiotics, and antibiotic disks plus 10µL of Fe_3O_4@ATB, respectively, according with their dimensions from CLSI, 2012. For these experiments there were used penicillin (P), streptomycin (S), erythromycin (E), kanamycin (K) and cefotaxime (CTX). The quantitative method for the minimal inhibitory concentration (MIC) assay of each Fe_3O_4@ATB consisted of two-fold microdilutions of nanoparticules stock solutions prepared in sterile saline were performed in liquid culture medium (nutrient broth) distributed in 96 multi-well plates. The concentration of each antibiotic adsorbed on magnetite surface was presented in previous published papers [15,16]. Each well was inoculated with 5 µL of microbial suspensions corresponding to a 0.5 McFarland density. Sterility, negative control wells (nutrient broth) and microbial growth, positive controls (inoculated nutrient broth) were used. The plates were incubated for 24 h at 37°C, and the influence of nanoparticles on the

planktonic cells growth in liquid medium was appreciated by measuring the A 600 nm of the obtained cultures. The MIC was considered as the last dilution of the tested compound which inhibited the microbial growth.

2.4. Assessment of the influence of Fe_3O_4@ATB on *S. aureus* biofilms. After performing MIC assay, the 96 well plates were emptied, washed 3 times with phosphate buffered saline, fixed with cold methanol and stained with violet crystal solution 1% for 30 min. The biofilm formed onto the plastic wells was resuspended in 30% acetic acid and the intensity of the stained suspension was assayed by measuring the absorbance at 490 nm [18]. The results interpretation was performed by comparing the obtained value of absorbance at 490 nm for strains treated with nanoparticles with those obtained for control strains (bacteria grown in standard conditions).

3. RESULTS SECTION

Magnetic nanoparticles consisting of magnetite (Fe_3O_4) possess unique characteristics that make them promising agents for antibacterial applications [19]. In this study we have investigated the influence of magnetic nanoparticles functionalized with antibiotics on the planktonic growth and biofilm formation by *S. aureus*. The results of the qualitative assay of the antimicrobial activity of the obtained nanosystems showed that the Fe_3O_4 nanoparticles clearly improved the activity of some of the tested antibiotics, as revealed by the increase of the growth zone inhibition diameter for penicillin, streptomycin and erithromycin, as compared with the antibiotic disks charged with the same antibiotic concentration (Figure 1).

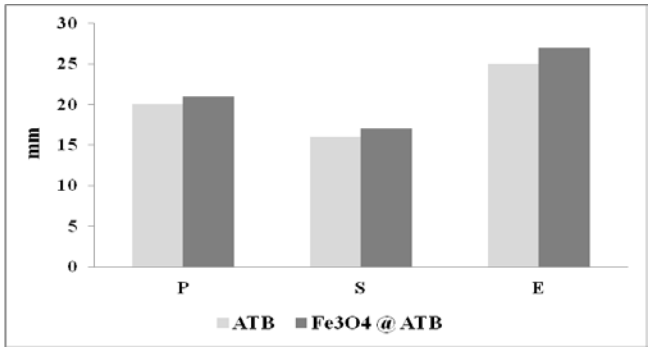

Figure 1: The growth inhibition diameter values of Fe_3O_4@ATB *versus* control antibiotics on *S. aureus* ATCC 29213

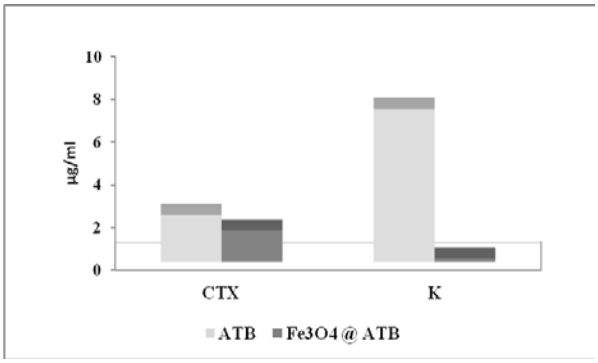

Figure 2: The MIC values of Fe_3O_4@ATB *versus* MIC values of control antibiotics on *S. aureus* ATCC 29213

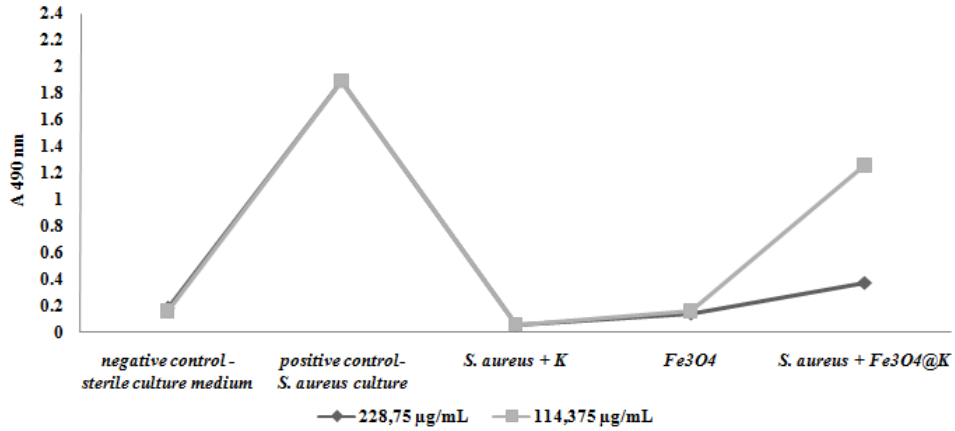

Figure 3: The effect of Fe_3O_4@K on *S. aureus* biofilm development

In the quantitative assay, the nanoparticles improved the antimicrobial activity of cefotaxime and kanamycin, as revealed by the significant decrease of the MIC value of these antibiotics (figure 2). The magnetic nanoparticles did not improve the efficacy of any of the tested antibiotics to prevent the *S. aureus* biofilms development. Figures 3-7 present the degree of *S. aureus* biofilm development in the presence of the first two higher concentrations of the tested nanostructures, as compared with the antibiotic control and magnetite control.

It is to be noticed that magnetite itself exhibited a superior anti-biofilm activity, as compared with the antibiotic loaded into the nanocarrier, being closer or even better, in case of kanamycin, than the antibiotic solution (Figure 3), demonstrating the utility of these metallic nanoparticles for the design on anti-biofilm surfaces.

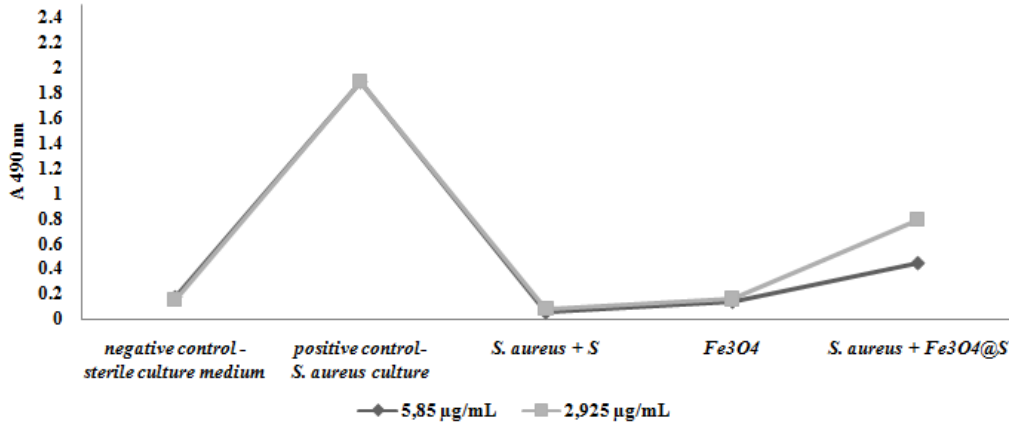

Figure 4: The effect of Fe_3O_4@S on *S. aureus* biofilm development

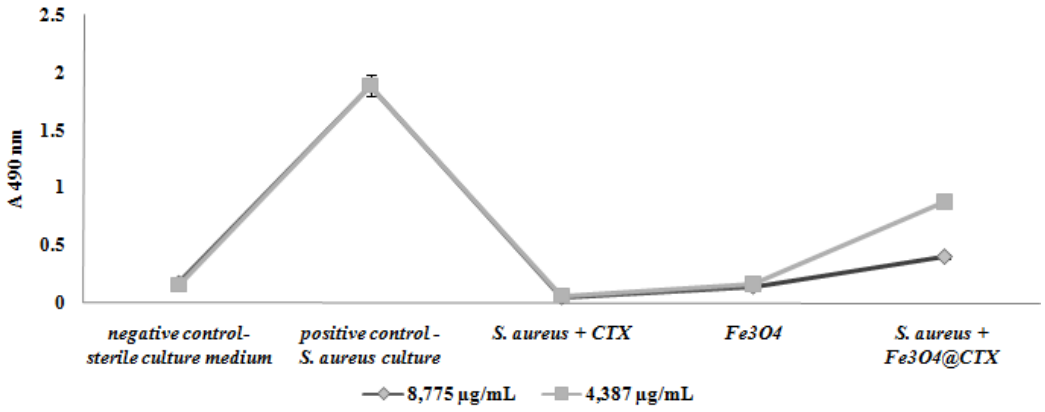

Figure 5: The effect of Fe_3O_4@CTX on *S. aureus* biofilm development

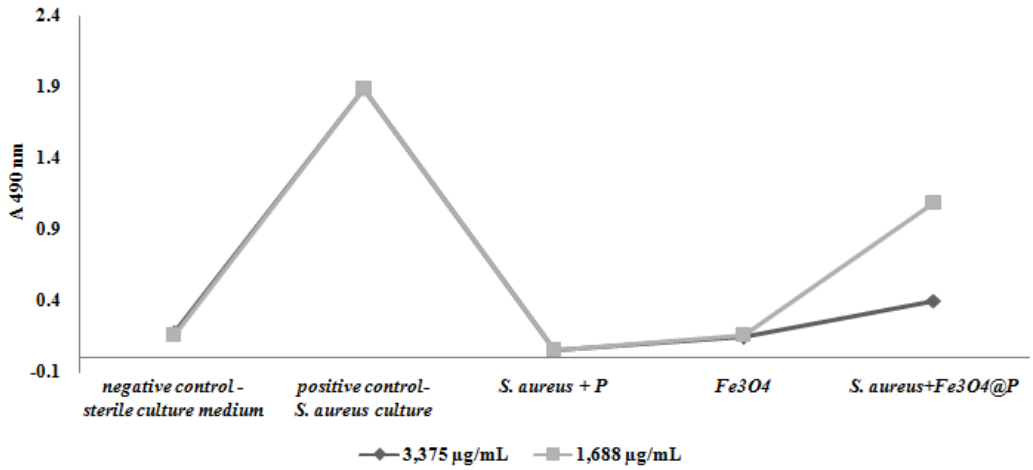

Figure 6: Thze effect of Fe_3O_4@P on *S. aureus* biofilm development

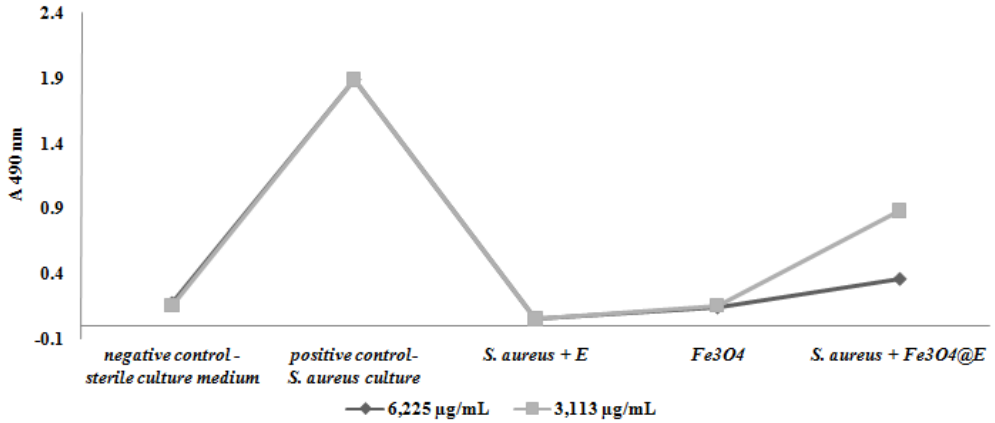

Figure 7: The effect of Fe_3O_4@E on *S. aureus* biofilm development

The alarming increase of *S. aureus* biofilm associated infections urged the search for the design and development of new and effective strategies to fight against this important pathogen. One of the most studied aspects of nanotechnology nowadays is their ability to offer the opportunity to fight microbial infections via the synthesis of nanoparticles. Magnetic nanoparticles consisting of magnetite (Fe_3O_4) possess unique characteristics that make them promising as carriers in biomedical utilizations. In terms of diagnosis they can be used both for *in vitro* and *in vivo* applications for example: in immobilization and detection of biomolecules [20], cell separation [21], purification [22] and gene transfer [23], and serve as a contrast agents in magnetic resonance imagining [24]. They can also be applied for drug delivery system in target therapy [25-30] and for hyperthermia treatment, due to the heat they produce in an alternating magnetic field [31]. Nanoparticles bind to bacterial cell walls causing membrane disruption through direct interactions or through free radical production [32]. Mammalian cells are able to phagocytose nanoparticles, and can subsequently degrade these particles by lysozomal fusion [33], reducing toxicity and free radical damage. This may allow for the selectivity of the same nanoparticle to promote tissue-forming cell functions, while also inhibiting bacterial functions that lead to infection.

Our results are clearly demonstrating that magnetite nanoparticles are improving the antimicrobial activity of investigated antibiotics, both against planktonic, as well as adherent *S. aureus* cells. The fact that the Fe_3O_4 functionalized with these antibiotics was much more efficient than the antibiotics alone against planktonic *S. aureus* cells, is demonstrating that the potentiating effect of the magnetic nanoparticles consists in binding to bacterial cell walls causing membrane disruption [34] and favouring the antibiotics activity. The fact that in case of the adherent bacterial cells, the Fe_3O_4@ATB efficiency was inferior to that of Fe_3O_4 itself or antibiotic solution is demonstating that magnetite nanoparticles could carry the antibiotic, but do not release it in an active form, that could diffuse and reach the biofilm cells. This could probably be due to the binding of antibiotics functional groups to the surface of the nanoparticles. The rest of the molecule is solvated in dispersion medium or in a fluid. The process, known to be entropic or steric, refers to the inhibition of particles aggregation by an entropic force, which appears when the particles are close to each other [35].

4. CONCLUSIONS

The use of nanoparticles is a growing new approach against biofilm-mediated, drug-resistant, and device centered infections. Nanoparticles offer an attractive alternative to conventional antibiotics in

the development of new-generation antibiotics due to their potent antimicrobial activity and unique mode of action. Our study investigated the ability of magnetic nanoparticles to improve the anti-bacterial activity of the current antibiotics against *S. aureus*, one of the most resistant opportunistic pathogens, both in planktonic and adherent state. The obtained results are suggesting that the magnetic nanoparticles may be considered effective antibiotics carrier, but a complete understanding of the way in which they selectively interact with different antibiotics and the bacterial cell is needed in order to obtain improved strategies for elimination of *S. aureus* biofilms on biomedical devices or human tissues.

5. ACKNOWLEDGMENT

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