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**ORIGINAL ARTICLE / ÖZGÜN MAKALE**

# INTERACTION OF *STAPHYLOCOCCUS AUREUS* WITH SOLID LIPID NANOPARTICLES

## *STAPHYLOCOCCUS AUREUS'UN KATI LİPİD NANOPARTİKÜLLER İLE ETKİLEŞİMİ*

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## ABSTRACT

**Objective:** *The ability of bacteria to develop resistance to existing antibiotics has made the search for new antimicrobials or antimicrobial formulations a matter of urgency. Among drug delivery systems, solid lipid nanoparticles are considered solution-oriented systems with advantages for targeted drug delivery.*

**Material and Method:** *In this study, the bacterial uptake of SLNs encapsulated with fluorescein into Staphylococcus aureus ATCC 29213 was investigated by a flow cytometry method to be a preliminary study for antibiotic improvement studies.*

**Result and Discussion:** *It was determined that ~60% of fluorescein-SLNs were taken into S. aureus ATCC 29213 cells within 1h. The results were found to be promising for future studies with antibiotics.*

**Keywords:** *Bacterial uptake, flow cytometry, Staphylococcus aureus, solid lipid nanoparticle*

## ÖZ

**Amaç:** *Bakterilerin mevcut antibiyotiklere direnç geliştirme yeteneği, yeni antimikrobiyallerin ya da antimikrobiyal formülasyonların araştırılmasının aciliyetini ortaya koymaktadır. İlaç taşıyıcı sistemler arasında, katı lipid nanopartiküller, hedeflenen ilaç uygulaması için avantajlara sahip, çözüm odaklı sistemler olarak kabul edilir.*

**Gereç ve Yöntem:** *Bu çalışmada, antibiyotik iyileştirme çalışmaları için bir ön çalışma olması amacıyla floresein yüklenmiş SLN'lerin Staphylococcus aureus ATCC 29213'e bakteri alımı akış sitometrisi yöntemiyle araştırıldı.*

**Sonuç ve Tartışma:** *Floresein-SLN'lerin ~%60'ının 1 saat içinde S. aureus ATCC 29213 hücrelerine alındığı belirlendi. Sonuçların gelecekte antibiyotiklerle yapılacak çalışmalar için umut verici olduğu bulundu.*

**Anahtar Kelimeler:** *Akış sitometresi, bakteriyel alım, katı lipid nanopartikül, Staphylococcus aureus*

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## INTRODUCTION

Antibiotic resistance is one of the global public health problems. The unconscious use of antibiotics causes the emergence of resistant bacteria and the spread of resistance among individuals. In addition, undesirable situations arise such as the fact that existing drugs are effective at higher doses, lose their effectiveness, and bacteria develop resistance to more than one different group of antibiotics [1, 2]. The failure of the strongest antibiotics to be effective against resistant strains highlights the urgency of the need to develop other alternative control agents. In order to maintain the treatment and prevent the formation of resistance, studies on developing new alternative drugs have gained importance. These alternative approaches include the development of active substances from plant sources [3], chemical modifications [4], inhibition of bacterial communication systems [5], use of phages [6], antimicrobial peptides, and nano-drug development technologies [7].

Nano-drug development technologies include nanoparticles which are carrier systems consisting of natural or synthetic polymers in nm sizes (10-1000 nm). In these systems, the drug can be dissolved or encapsulated [8, 9]. Solid lipid nanoparticles (SLNs) are colloidal carrier systems developed as an alternative to existing conventional carriers such as polymeric nanoparticles, liposomes, and nanoemulsions. SLNs are new generation nano-sized lipid emulsions in which a liquid lipid is replaced with a solid lipid and since the particles are completely metabolized by the body, it is considered quite safe for administration. In addition to their large surface area, tolerability, stability, scaling-up feasibility, these nanoparticles improve the performance of pharmaceuticals with their high drug loading capacity and phase interactions [10].

One of the main obstacles to the elimination of target bacteria in the host during treatment is considered to be the low uptake of antibiotics into infected cells. This situation is also related to the intracellular resistance of the bacteria and creates the need for studies to increase the uptake of antibiotics into the cell [11, 12].

*Staphylococcus aureus* (*S. aureus*) is a Gram-positive bacterium that is one of the major human pathogens. *S. aureus* causes a wide range of infections like bacteremia, infective endocarditis, device-related, skin and soft tissue, pleuropulmonary infections [13]. It has also many virulence factors associated with these infections; enzymes such as hemolysin, leukocidin, protease; enterotoxins, exfoliative toxins, etc. toxins, and immune-modulatory factors [14]. *S. aureus* is among the bacteria known for its ability to develop resistance to antibiotics. Infections initiated by antibiotic-resistant strains can generate epidemic waves [15].

To provide a solution to the different problems listed above and to improve the therapeutic potential of drugs, this study aimed to investigate the bacterial uptake of SLNs encapsulated with fluorescein in *S. aureus* ATCC 29213. SLNs were prepared and characterized in terms of size,

polydispersity index (PI), and zeta potential. Moreover, comprehensive microscopy and flow cytometry studies showed the bacterial uptake of SLNs.

## MATERIAL AND METHOD

### Materials and reagents

Fluorescein and Pluronic F127 was purchased from Sigma-Aldrich (USA). Compritol ATO 888 was gifted from GATTEFOSE (France). In addition, bacterial media were purchased from Merck Life Science (Germany). Tryptic soy agar (agar; 15 g/L, casein peptone (pancreatic); 15 g/L, sodium chloride; 5 g/L, soya peptone; 5 g/L), and tryptic soy broth (casein peptone; 17 g/L, dipotassium hydrogen phosphate; 2.5 g/L, glucose; 2.5 g/L, sodium chloride; 5 g/L, soya peptone; 3 g/L) were used in bacterial production. All other chemicals and reagents used in the study but not included in the text were used as an analytical grade.

### Preparation of solid lipid nanoparticles

Hot homogenization and ultrasonication methods were used to prepare the nanoparticles. Compritol ATO 888 (150 mg) and Pluronic F127 (100 mg) were added in a wide-mouth glass container as lipid phase and heated to 70°C by a heating mantle (IKA, Germany). 5 mg fluorescein was dissolved in ultrapure water and heated to 70°C. The oil phase was poured into the fluorescein solution under magnetic stirring at 300 rpm for 1 minute to form an oil in water emulsion, and then further probe sonicated for 1 minute (Bandelin Sonoplus, Germany), with an amplitude of 50 % to form a nanoemulsion. The nanoparticles were purified by dialysis (12-14 kDa) against ultrapure water for 24 h at 3 times. The SLNs were lyophilized (Christ Gama 2-20, USA). The control SLN was prepared with a similar method without adding fluorescein.

### Determination of the size of the particle, polydispersity index, and zeta potential

The photon correlation spectroscopy (PCS) using Zetasizer Nicomp Z3000 at 25°C was used to measure the size of the particle, PI, and zeta potential of fluorescein loaded SLNs. To remove the bubbles and disperse aggregates the samples were ultrasonicated for 5 seconds at 0°C to suspend in distilled water. For particle size and PI, the concentration of samples was 2.7 mg/mL. To achieve the optimal kilo counts per second of 20 to 400 for measurements, the sample concentration was 0.3 mg/mL for zeta potential determinations.

### Bacterial strain

*S. aureus* ATCC 29213 was tested as a bacterial strain. Tryptic soy agar and broth medium were used for bacterial production and the study was carried out with a 24-hour fresh bacterial culture.

**Bacterial uptake of nanoparticles**

To exhibit the bacterial uptake of SLNs, fluorescent microscopy examination and flow cytometry analysis were carried out. At the exponential phase of *S. aureus* ATCC 29213, the calculated amount of SLNs was added to 0.1 mL bacterial suspension and incubated for 1 and 4 hours at 37°C. After 1 and 4 h incubation, the bacterial pellet and culture supernatant was separated by centrifugation at 8000 rpm. The pellet of *S. aureus* ATCC 29213 was washed with sterile distilled water 3 times to dispose of free SLNs. Then the pellet was suspended in 1 mL of sterile distilled water. The CYTOFlex cytometer (Beckman Coulter, USA) was used to observe the fluorescent intensity of *S. aureus* cells. Green fluorescence was collected through a 488 nm blocking filter, a 525/40 BP fluorescent channel. Thereabout ten thousand cells of each sample were analyzed. Data were evaluated and histograms were generated and interpreted using CytExpert Software. In addition, to detect the position of the fluorescein-SLNs in bacteria, the cells were observed under an inverted fluorescent microscope (Leica, Germany) at 40x magnification, and images were taken [8].

## RESULT AND DISCUSSION

In this study, a flow cytometry method for the detection of bacterial uptake of nanoparticles has been investigated. The fact that nanoparticle uptake into bacteria can be evaluated over several generations constitutes the originality of this study. Flow cytometry provides rapid, multi-parameter analysis, reducing false-negative results, obtaining accurate statistical data, and has advantages compared to transmission electron microscopy.

Firstly, some characterization studies were performed for fluorescein-loaded SLNs. Particle size and distribution were found 183.6 ± 0.45 nm and 0.296 ± 0.01 respectively. The Zeta potential of SLNs was detected -8.33 mV. According to the results, we achieved to obtain small particles within a narrow particle range.

The correlation of the forward (FSC), and side scatter (SSC) intensity are well established [16, 17] with the cellular size, granularity of cells, and cellular mass. According to the differential forward, and side scatter properties, locating bacteria in the FSC-SSC dot plot is constituting the aim of the experiment.

In the control histogram of *S. aureus* ATCC 29213 cells (Figure 1a), there was no fluorescence detected. Otherwise, significant fluorescence was determined in the flow cytometry histogram (Figure 1b-1d) of *S. aureus* ATCC 29213 cells treated with fluorescein and fluorescein-loaded SLNs. Histograms further revealed that fluorescein-SLNs were more efficiently up taken (59.28% and 99.48% for 1h and 4h, Figure 1b, 1d) than fluorescein solution (18.02% and 25.23% for 1h and 4h, Figure 1c, 1e).

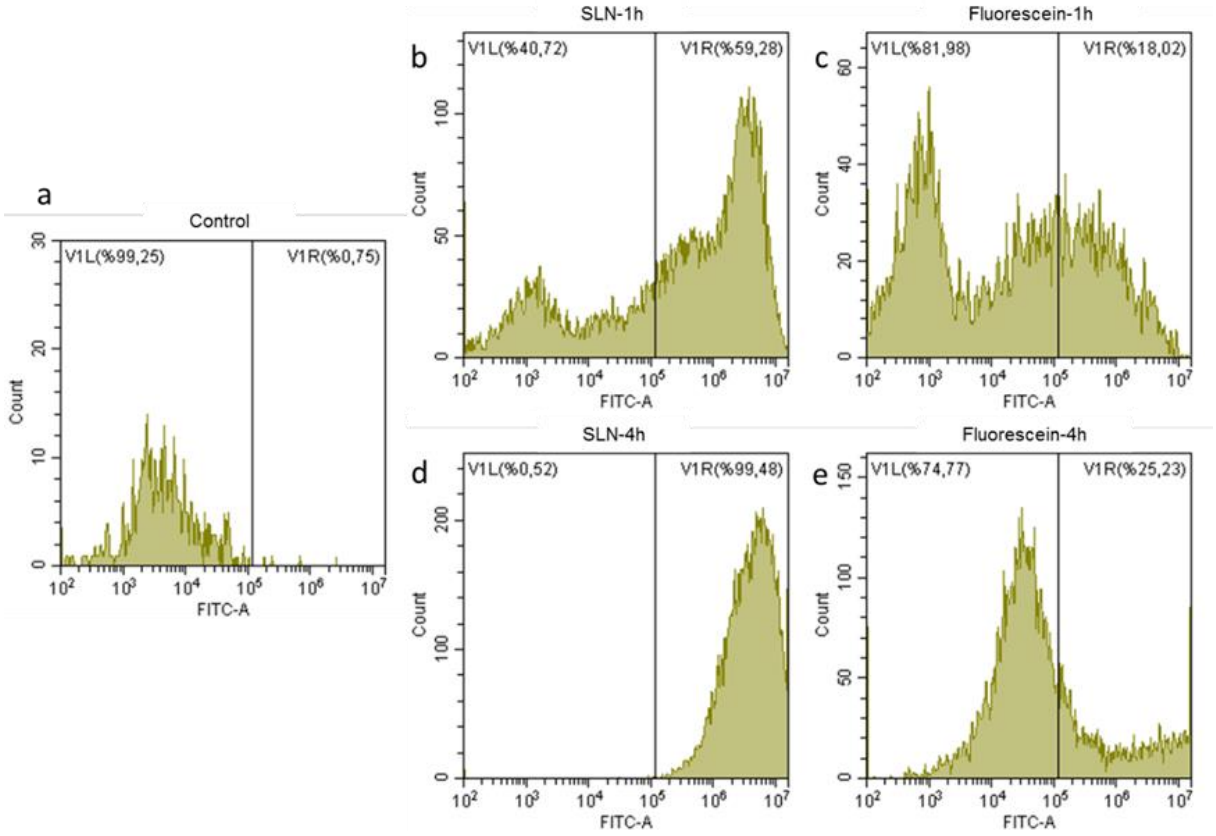


**Figure 1.** Bacterial uptake of *S. aureus* ATCC 29213

The fluorescein-SLNs entreated bacterial samples were analyzed by fluorescent microscopy to characterize the uptake of nanoparticles by *S. aureus* ATCC 29213. The images indicated by the figures below were obtained after 4 hours of incubation. Figure 2a shows the bright image of *S. aureus* ATCC 29213. Figure 2b points out the fluorescent images of *S. aureus* ATCC 29213 cells, which underlined that just a few bacterial cells internalized the fluorescein solution in a low frequency. Whereas Figure 3b shows the higher amount of bacterial cells have uptaken fluorescein-SLNs. The resulting data show that SLNs are efficient systems for bacterial cell uptake.

In this study, bacterial uptake of fluorescein loaded-SLNs was determined by a flow cytometry method. It constitutes a preliminary study for the use of SLNs and flow cytometry analysis as an alternative method in the fight against antimicrobial resistance in the improvement studies of different antimicrobials or antimicrobial formulations.

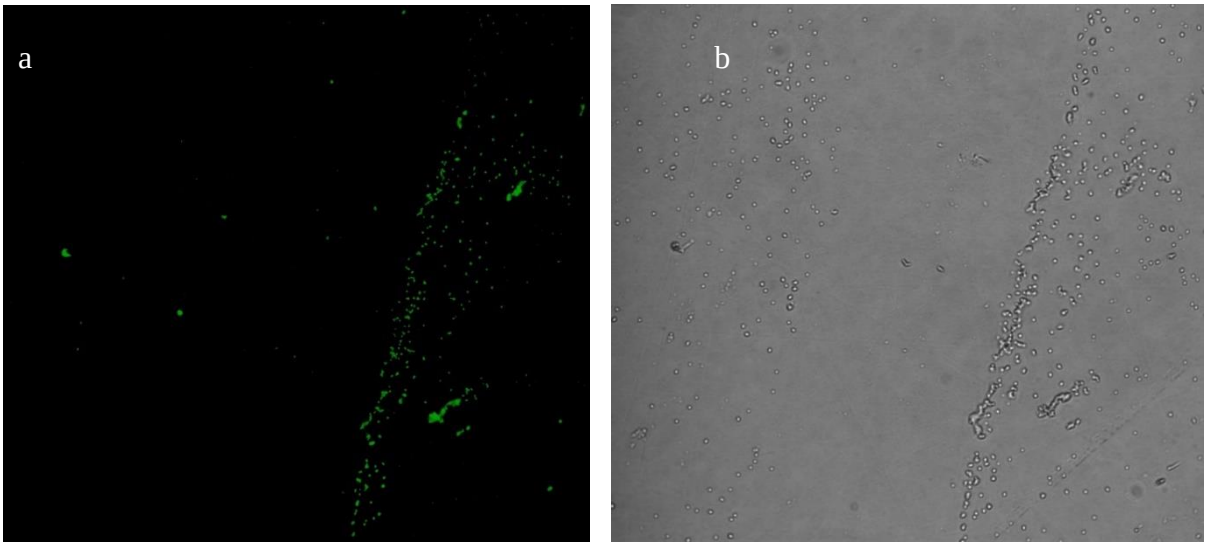


**Figure 2a.** The bright image of *S. aureus* ATCC 29213, **Figure 2b.** The fluorescent images of *S. aureus* ATCC 29213 cells treated with fluorescein solution

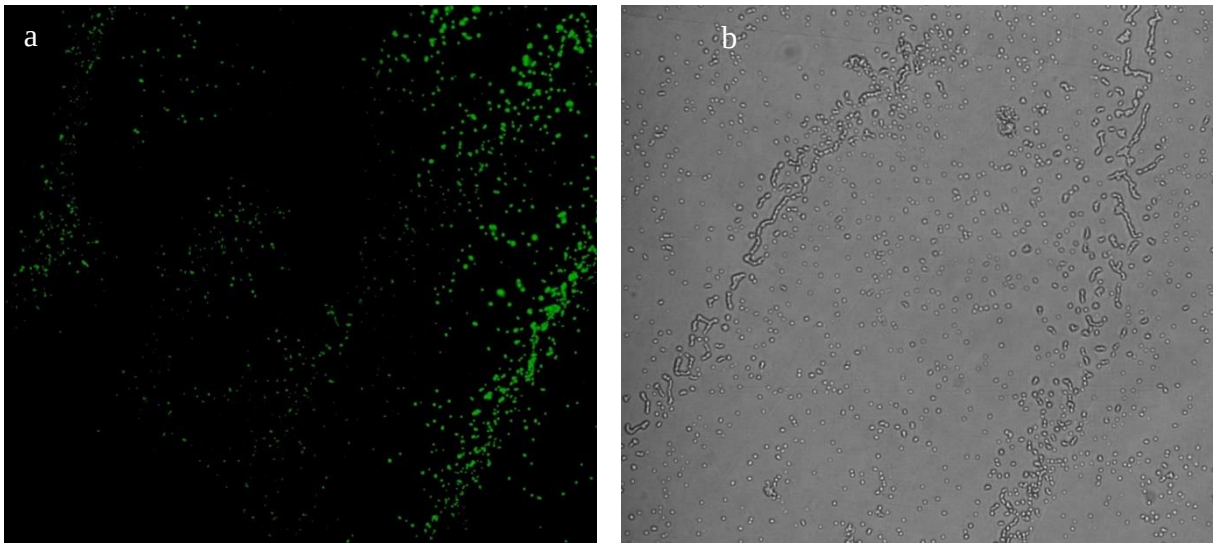


**Figure 3a.** The bright image of *S. aureus* ATCC 29213 treated with fluorescein-SLNs, **Figure 3b.** The fluorescent images of *S. aureus* ATCC 29213 cells treated with fluorescein-SLNs

## AUTHOR CONTRIBUTIONS

Concept: *M.E.K.*; Design: *M.E.K.*; Control: *M.E.K.*; Sources: *M.E.K.*; Materials: *M.E.K.*; Data Collection and/or Processing: *M.E.K.*; Analysis and / or Interpretation: *M.E.K.*; Literature Review: *M.E.K.*; Manuscript Writing: *M.E.K.*; Critical Review: *M.E.K.*; Other: *M.E.K.*

## CONFLICT OF INTEREST

The authors declare no conflict of interest.

## ETHICS COMMITTEE APPROVAL

The authors declare that the ethics committee approval is not required for this study.

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