Nanomed. J. 10(1): 41-46, Winter 2023

RESEARCH PAPER

Enhanced antibacterial activity in skin exudates isolated MDR *Staphylococcus aureus* by γ-Al_2O_3 nanoparticles

Bahareh Panahi [1], Mahkameh Soleimani Maygouni [2], Davood Zaeifi [1, 3]*

[1]Biology Department, Faculty of Biological Sciences, Tehran North Branch of Islamic Azad University, Tehran, Iran
[2]Department of Biology, Islamic Azad University of Medical Sciences, Tehran, Iran
[3]Department of Cell and Molecular Biology, School of Biology, University of Tehran, Tehran, Iran

ABSTRACT

Objective(s): *Staphylococcus aureus* is one of the most common causes of infections affecting the skin and soft tissues, which causes many types of syndromes, including skin and soft tissue infections in humans. The quick occurrence of resistance to many antimicrobial substances and severe infections requires long-term intravenous administration of beta-lactamase-resistant Penicillin.

Materials and Methods: The antimicrobial activity of γ-Al_2O_3 nanoparticles (NPs) against 20 clinical samples of *S. aureus* isolated from skin exudates compared with the standard ATCC 25923 strain investigated alone and in synergy with an antibiotic showed resistance. The most resistant isolates were selected based on being positive for MepA and Kirby and Bauer disc diffusion method. Minimum inhibitory concentration (MIC) of γ-Al_2O_3 NPs against *S. aureus* was determined within 0-360 min treatment time. Then, the double-disc synergy test (DDST) method was performed for semi-sensitive and antibiotic-resistant strains to evaluate the probable inhibitory effect in synergy form.

Results: The selected isolate expressed the MepA gene, showed the highest susceptibility reaction against γ-Al_2O_3 NPs in Z=78.125 ml/μg^{-1} and Z=156.25 ml/μg^{-1}, and the process continued by performing the best ratio of NPs on semi-sensitive and also resistance antibiotic in synergy with NPs for the bacteria strains. The synergy of γ-Al_2O_3 NPs and Tetracycline, Oxacillin, and Ceftazidime showed higher sensitivity compared to using antibiotics alone.

Conclusion: The results of this study demonstrate that γ-Al_2O_3 has a strong antimicrobial effect and can enhance the properties and characteristics of antibacterial potency in synergy or developed synthetic functionalized NPs with antibiotics.

Keywords: *MepA gene; Staphylococcus aureus; Skin exudates; γ-Al_2O_3 nanoparticles*

How to cite this article
Panahi B, Soleiman Meygouni M, Zaeifi D. EEnhanced antibacterial activity in skin exudates isolated MDR Staphylococcus aureus by γ-Al_2O_3 nanoparticles. Nanomed J. 2023; 10(1): 41-46. DOI: 10.22038/NMJ.2022.68234.1732

INTRODUCTION

Staphylococcus aureus is one of the most common causes of bacteremia, pneumonia, bloodstream, cellulitis, and osteomyelitis, and infections that affect the skin and soft tissues are also significant causes of hospital-acquired (nosocomial) infection [1-3]. These Gram-positive spherical bacteria are commonly found in the irregular, grape-like cluster, non-motile, and do not form spores. The prevalence of this bacteria is high in Iran, particularly the prevalence of methicillin-resistant *S. aureus* is much more than in others. This bacteria can be spread through the nose, skin, and eyes. Approximately 20-50% of people are carriers of *S. aureus* in their noses [4, 5]. Heat-resistant enterotoxin of *S. aureus* is the most common cause of food poisoning, and the mortality rate caused by this bacteria is around 20-30%. Unfortunately, these bacteria become resistant to antibiotics over time [5, 6].

S. aureus is Coagulase-positive, which separates it from other species; also, catalase is distinguished from streptococci by producing catalase. Plasmid-mediated β-lactamase is produced in this bacteria, resulting in resistance to most penicillins such as Ampicillin, Ticarcillin (penicillin G and Piperacillin),

* *Corresponding author: Email: d.zaeifi@ut.ac.ir; d.zaeifi@iau-tnb.ac.ir*
Note. This manuscript was submitted on August 1, 2022; approved on October 8, 2022

and other similar drugs [5].

The quick occurrence of resistance to many antimicrobial substances is one of the difficulties in treating Staphylococcal, as well as the lack of penetration of drugs into the centre of the necrotic lesion. Severe infections caused by *S. aureus* require long-term intravenous administration of beta-lactamase-resistant Penicillin [7, 8]. In cases where Staphylococcus is resistant to Penicillin, Vancomycin is often used. In recent years the increase in the vancomycin MIC among the majority of MRSA strains isolated from hospital patients has made physicians search for alternative treatments. Alternative agents for treating bacteremia and endocarditis caused by these strains are recent antibiotics such as Daptomycin, Linezolid, and Quinupristin/Dalfopristin [3, 5, 8].

Susceptibility or resistance to antibiotics is varied among different strains of this bacteria. On average, *S. aureus* is resistant to Ampicillin, is intermediate to Cotrimoxazole, Tetracycline, and Chloramphenicol, and is sensitive to Ciprofloxacin, Erythromycin, and Gentamicin (9].

Sequence analysis revealed that MepA gene is integrated into the mepRAB operon, encoded by the chromosomal gene mepA, and it was the first multidrug transporter from the MATE family to be described in *S. aureus*, which reveals the MDR phenotypes (10].

Researchers are trying to find more appropriate therapeutic strategies for this disease. Hence, nano-materials find widespread applications every day and find their way into various parts of our life, and they can be introduced as a solution. Nanotechnology, with a wide variety of products, has many applications in biology and medicine. These materials are also racing towards promoting industrial production, especially the new generation of drugs. Until now, few studies on the effect of γ-Al_2O_3 NPs multidrug resistance strains of *S. aureus* have been carried out. γ-Al_2O_3 NPs not only in the cell membrane but also penetrate the bacteria causing irregular holes and granules. According to studies, the interaction of γ-Al_2O_3 with cells, tissues, and organs is effective and can be used at the appropriate dose for therapeutic effects [11]. This study aims to find a way to prevent drug resistance and use lower doses of antibiotics to treat infections caused by these bacteria.

MATERIALS AND METHODS

The reference strain, *S. aureus* (ATCC 25923), was used as a control strain in all steps for comparison purposes. The current study was approved in 2021 by the research ethics committee of Tehran University of Medical Sciences, and participants provided written informed consent voluntarily.

Sample preparation

A total of 20 clinical isolates of *S. aureus* were prepared from skin exudates such as pus, wounds, burns, etc. The isolates were identified as *S. aureus* morphologically on culture, by Gram staining and biochemical tests, and were cultured on mannitol salt agar plates (7 to 9% salt) (Merck, Germany).

Kirby-bauer susceptibility (sensitivity) test

The clinical-positive specimens of *S. aureus* were cultured on Mueller Hinton agar plates (Merck, Germany), and the Kirby Bauer disc diffusion method was performed using various antibiotic discs declared in (Table 1) (Mast Group Ltd Company, UK). The cultures were incubated for 24 h at 37 °C supplemented with 5% CO_2 (semi-aerobic condition). Following measuring the inhibition zones, the isolate which showed resistance to multiple antibiotics was selected for further study.

DNA extraction and molecular identification

The genomic DNA of *S. aureus* isolates was extracted using QIAamp DNA Mini Kit supplemented by the manufacturing company (Qiagen, Germany), and conventional PCR was used for detection of the specific genes, 16S rRNA gene (F5-TCACCGTAGCATGCTGATCT-3, R5-AGGTGGGGATGACGTCAAAT-3) for identification of the Staphylococcus genus and MepA gene (F5-GCAGTTATCATGTCTATCGGCG-3, R5-TGCACCTTGTAAAATGGCCA -3) in selected *S. aureus.*

Preparation of nanoparticle suspension

Commercial γ-Al_2O_3 NPs with purity over 99.7% and a 10-30 nm size range were purchased from US NANO and were prepared based on Fathi et al. (2016) and Shahbazi et al. (2018). The Kirby Bauer disc diffusion and NPs suspension were performed simultaneously to prevent possible errors [12, 13].

Microbial suspension preparation

Bacterial colonies from mannitol salt agar medium were resuspended in 15 mL of the sterile PBS for achieving CFU=1-1.5×10^8 using a UV/

visible spectrophotometer (UNICO-2100, US) at 620 nm with an absorption rate in the range of 0.08-0.1 [14].

MIC test

The MIC was defined by serial dilutions using the standard method suggested by the National Committee of the Clinical Laboratories Standards Institute (CLSI) in brain heart infusion (BHI) broth medium (CONDALAB/ pronadisa/ Spain) using the tube method. In brief, 2 ml of NPs added to the first tube, which contained 2 ml of BHI medium; after pipetting 2 ml of the first was added to the second tube and went on to tube 12^{th}; The remained 2 ml from the last tube was dropped out. Then 1 ml (15] of microbial suspension was added to each tube and incubated at 37°C for 360 min and 60 rpm shaking rate. Aliqouqtes of 100 µl were taken every 60 min from each tube, spread on plates, and incubated at 37°C overnight for colony formation. The next day, the former colonies were counted, and survival rates were calculated [12, 13].

Double disc synergy test (DDST)

Sterile blank discs were impregnated with the selected MIC concentrations of the nanoparticle suspension and allowed to dry at room temperature for 24 h [16].

The antibiotics (Table 1) to which *S. aureus* showed resistance or lower susceptibility were used for DDST in Mueller Hinton agar. The cultures were incubated at 37°C for 18 h, and the zone of inhibition for each disc was recorded [12, 13].

RESULTS

Kirby-bauer susceptibility test

The selected *S. aureus* isolate was resistant to Amikacin, Oxacillin, Penicillin, and Ampicillin antibiotics; and semi-sensitive to Nalidixic acid, Ceftazidime, Trimethoprim & Sulfamethoxazole antibiotics (Table 1).

Detection of MepA

The selected sample expressed the MepA gene in the expected size at 240bp, which shows that it is positive for the MDR phenotype (Fig. 1).

Preparation of nanoparticle suspension

The XRD and TEM confirmed the purity and size of applied γ-Al_2O_3 nanoparticles (Fig. 2). The results obtained through the γ-Al_2O_3-NPs XRD application validated the purity of NPs, as no impurity except oxygen was detected. Also, XRD images obtained for the nanoparticle showed peaks, indicating the precise crystal structure of the NPs, respectively.

MIC test

The MIC value for γ-Al_2O_3-NPs against the clinical isolate of *S. aureus* and ATCC strain showed

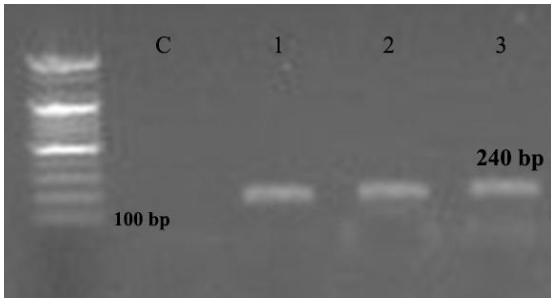

Fig. 1. Agarose gel electrophoresis of PCR products MepA in size (240)bp; lanes 1-3: repeated PCR products of MepA gene; lane C: Negative control

Table 1. Sensitivity of the *S. aureus* to the applied antibiotics and in synergy with γ-Al_2O_3 NPs

		ATCC25923	Clinical Sample	
Antibiotic	mg	Zone diameter (mm) mast disc	Zone diameter (mm) mast disc	Zone diameter (mm) DDST
Nalidixic acid	30	15	12	12
Amikacin	30	0	0	0
Tetracycline	30	17	0	15
Oxacillin	1	0	0	12
Penicillin	10	0	0	0
Ampicillin	25	0	0	0
Ceftriaxone	30	30	28	(NT)*
Ceftazidime	30	17	12	15
Trimethoprim & Sulfamethoxazole	25	20	15	15

* (NT) – not tested

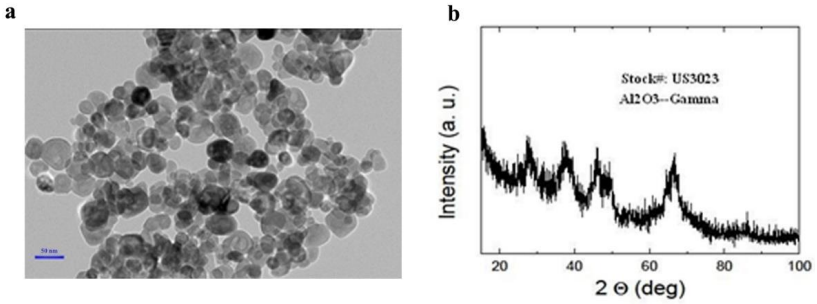

Fig. 2. The shape, size, and purity of γ-Al_2O_3 NPs: a) TEM high-resolution picture of γ-Al_2O_3 NPs show the morphologies and the corresponding particle size distributions over the prepared volume. b) X-Ray graph of γ-Al_2O_3 No diffraction patterns originating from any impurities detected

78.125 ml/µg^{-1} and 156.25 ml/µg^{-1}, respectively. The sensitivity coefficient of both strains to the nanoparticle at different sampling periods was calculated (Fig. 3).

DDST Susceptibility test

The γ-A_2O_3 NPs applied with Tetracycline and Oxacillin overcame resistance. A slight increase in the inhibition zone was observed for Ceftazidime. No change in susceptibility to Nalidixic acid and Trimethoprim & Sulfamethoxazole in synergy form

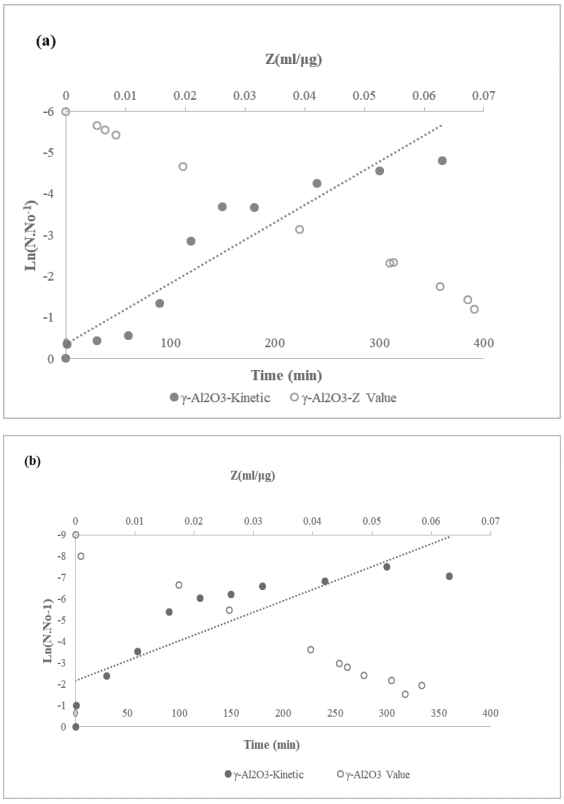

Fig. 3. Coefficient sensitivity of *S. aureus* population according to the exposure time. (a), a clinical sample, (b) in standard strain

was observed (Table 1).

DISCUSSION

The Transmission Electron Microscopy (TEM) images showed the shape and size range of the NPs; γ-A_2O_3 NPs had a relatively regular spherical shape and a 10-30 nanometer range size [17-19]. Reducing the NPs size changed their structural and physicochemical features, thus, enabling them to reach more inside the bacteria [20]. A decrease in the NPs size level results in an increased specific surface area, and the rising of the reactive group numbers on NPs surfaces have already been reported [21]. The increase of the surface-active groups may prepare more active sites for reactive oxygen species (ROS), including superoxide, hydrogen peroxide, and hydrocele radicals that lead to oxidative stress [22].

According to Fig. 3, increased NPs concentration enhanced the inhibitory property of the NPs against *S. aureus*. An increase in NPs concentration for defining MIC caused a slight decrease in the bacteria's sensitivity coefficient. In other words, γ-Al_2O_3 NPs did not kill the bacteria in the tested strains. However, the study results indicated that growth inhibition was not synergistically effective besides Nalidixic acid, Trimethoprim & Sulfamethoxazole (Table 1).

Researchers evaluated the γ-Al_2O_3 NPs bacterial cell surface adsorption, and the results indicated that the chemical composition of environmental water significantly influences electrostatic interactions between NPs and bacteria membranes [17]. In another study, NPs size was directly correlated with membrane and binding receptor activation and protein expression [23].

The γ-Al_2O_3 nanoparticles' antibacterial activity functionalized by massive structural patterns

and ionic presence makes it a good candidate for pharmacophore due to more interaction or penetration in the cause of distance between the positively charged groups and the NPs with the bacterial cell membrane (lipopolysaccharide) and inactivating them [24-26].

Literature suggests MepA be associated with an MDR phenotype, with low-level resistance to quaternary ammonium compounds and dyes like tigecycline, ethidium bromide, chlorhexidine, and almost antibiotics from the class of glycylcyclines. The fluoroquinolones ciprofloxacin and norfloxacin were shown to be weak substrates of MepA [27].

According to the above explanations, a higher concentration of NPs due to interfering with electrostatic force would not be an appropriate strategy for overcoming. These results conducted that γ-Al_2O_3 in different concentrations presented bacteriostatic effects against the bacteria samples but, at higher defined MIC, could be used as a source of energy for metabolism and growth. This study discovered the new functionalized form of nanoparticles in synergy with antibiotics, which can benefit future drug design and limit drug resistance in microorganisms. Because of less hemolytic potency (depending on the size and shape) in comparison with other oxide nanoparticles [28], γ-Al_2O_3 can be considered a good candidate for bacterial elimination that causes bacteremia, pneumonia, bloodstream, cellulitis, and osteomyelitis, and infections that affect the skin and soft tissues and nosocomial infections in the medical domains. Further studies are suggested for its pharmacophoric potency in binding or inactivating bacterial metabolic factors.

CONCLUSION

The γ-Al_2O_3 NPs suspensions had inhibitory effects against the kinetic activity of this bacteria. Therefore its inhibitory effects in combination with antibiotics like oxacillin and Tetracycline could be an excellent candidate to eliminate bacteria for nosocomial or in functionalized form could facilitate the effectiveness of the relevant drug in the medical domains.

ACKNOWLEDGMENTS

This research received no specific grant from any funding agency in the public, commercial, or not-for-profit sectors.

CONFLICTS OF INTEREST

The authors declare that they have no conflicts of interest.

REFERENCES

1. DeLeo FR, Otto M, Kreiswirth BN, Chambers HF. Community-associated meticillin-resistant *Staphylococcus aureus*. Lancet. 2010;375(9725):1557-1568.
2. Fridkin SK, Hageman JC, Morrison M, Sanza LT, Como-Sabetti K, Jernigan JA, et al. Methicillin-resistant *Staphylococcus aureus* disease in three communities. N Engl J Med. 2005;352(14):1436-1444.
3. Talan DA, Krishnadasan A, Gorwitz RJ, Fosheim GE, Limbago B, Albrecht V, et al. Comparison of *Staphylococcus aureus* from skin and soft-tissue infections in US emergency department patients, 2004 and 2008. Clin Infect Dis. 2011;53(2):144-149.
4. Mohammadi A, Ebrahimi A, Nemati S. Bacterial and Fungal Contamination of Elevator Buttons in University Schools of Isfahan University of Medical Sciences, Isfahan, Iran. Health Scope. 2016;5(4):e34428.
5. Morse S, F. Brooks G, C. Carroll K, S. Butel J, Mietzner T. Jawetz, Melnick & Adelberg's Medical Microbiology. 26 ed2013.
6. den Reijer PM, Lemmens-den Toom N, Kant S, Snijders SV, Boelens H, Tavakol M, et al. Characterization of the humoral immune response during *Staphylococcus aureus* bacteremia and global gene expression by *Staphylococcus aureus* in human blood. PLoS One. 2013;8(1):e53391.
7. Boles BR, Horswill AR. agr-Mediated Dispersal of *Staphylococcus aureus* Biofilms. PLoS Pathog. 2008;4(4):e1000052.
8. Kobayashi SD, Malachowa N, DeLeo FR. Pathogenesis of *Staphylococcus aureus* Abscesses. Am J Pathol. 2015;185(6):1518-1527.
9. Kitara LD, Anywar AD, Acullu D, Odongo-Aginya E, Aloyo J, Fendu M. Antibiotic susceptibility of *Staphylococcus aureus* in suppurative lesions in Lacor Hospital, Uganda. Afr Health Sci. 2011;11 Suppl 1:S34-39.
10. Kaatz GW, McAleese F, Seo SM. Multidrug resistance in *Staphylococcus aureus* due to overexpression of a novel multidrug and toxin extrusion (MATE) transport protein. Antimicrob Agents Chemother. 2005;49(5):1857-1864.
11. Ansari MA, Khan HM, Khan AA, Pal R, Cameotra SS. Antibacterial potential of Al_2O_3 nanoparticles against multidrug resistance strains of *Staphylococcus aureus* isolated from skin exudates. J Nanoparticle Res. 2013;15(10):1970.
12. Fathi Azar Khavarani M, Najafi M, Shakibapour Z, Zaeifi D. Kinetics activity of Yersinia Intermedia Against ZnO Nanoparticles Either Synergism Antibiotics by Double-Disc Synergy Test Method. Iran J Biotechnol. 2016;14(1):39-44.
13. Shahbazi E, Moreshedzadeh F, Zaeifi D. Bacteriostatic Potency of Fe2O3 Against Enterococcus Faecalis in Synergy with Antibiotics by DDST Method. Avicenna J Med Biotechnol. 2019;11(2):176-179.
14. Ruparelia JP, Chatterjee AK, Duttagupta SP, Mukherji S. Strain specificity in antimicrobial activity of silver and copper nanoparticles. Acta Biomater. 2008;4(3):707-716.
15. Food U, Administration D. Division of Antiinfective and Ophthalmology Drug Products (HFD-520)—Microbiological data for antibacterial drug products—development, analysis, and presentation. FDA; 2005.
16. Balouiri M, Sadiki M, Ibnsouda SK. Methods for *in vitro* evaluating antimicrobial activity: A review. J Pharm Anal. 2016;6(2):71-79.
17. Jastrzębska AM, Karwowska E, Olszyna AR, Kunicki A. Influence of bacteria adsorption on zeta potential of Al_2O_3 and Al_2O_3/Ag nanoparticles in electrolyte and drinking water environment studied by means of zeta potential.

SURF COAT TECH. 2015;271:225-233.
18. Jastrzebska AM, Karwowska E, Olszyna AR, Kunicki AR. Comparative Assessment of Antimicrobial Efficiency of Ionic Silver, Silver Monoxide, and Metallic Silver Incorporated onto an Aluminum Oxide Nanopowder Carrier. J Nanosci. 2013;2013:12.
19. Stengl V, Houšková V, Bakardjieva S, Murafa N, Maříková M, Opluštil F, et al. Zirconium doped nano-dispersed oxides of Fe, Al and Zn for destruction of warfare agents 2010. 1080–1088.
20. Lorenz CS, Wicht A-J, Guluzada L, Luo L, Jäger L, Crone B, et al. Nano-sized Al_2O_3 reduces acute toxic effects of thiacloprid on the non-biting midge Chironomus riparius. PLoS One. 2017;12(5):e0176356.
21. Nel AE, Mädler L, Velegol D, Xia T, Hoek EMV, Somasundaran P, et al. Understanding biophysicochemical interactions at the nano–bio interface. Nat Mater. 2009;8:543.
22. Li X, Zhou S, Fan W. Effect of Nano-Al_2O_3 on the Toxicity and Oxidative Stress of Copper towards Scenedesmus obliquus. Int J Environ Res Public Health. 2016;13(6):575.
23. Jiang W, Kim BYS, Rutka JT, Chan WCW. Nanoparticle-mediated cellular response is size-dependent. Nature Nanotechnology. 2008;3:145.
24. Raghupathi KR, Koodali RT, Manna AC. Size-Dependent Bacterial Growth Inhibition and Mechanism of Antibacterial Activity of Zinc Oxide Nanoparticles. Langmuir. 2011;27(7):4020-4028.
25. Wu D, Chen Z, Cai K, Zhuo D, Chen J, Jiang B. Investigation into the antibacterial activity of monodisperse BSA-conjugated zinc oxide nanoparticles. CURR APPL PHYS. 2014;14(11):1470-1475.
26. Yeaman MR, Yount NY. Mechanisms of Antimicrobial Peptide Action and Resistance. Pharmacol Rev. 2003;55(1):27.
27. McAleese F, Petersen P, Ruzin A, Dunman PM, Murphy E, Projan SJ, et al. A novel MATE family efflux pump contributes to the reduced susceptibility of laboratory-derived *Staphylococcus aureus* mutants to tigecycline. Antimicrob Agents Chemother. 2005;49(5):1865-1871.
28. Vinardell MP, Sordé A, Díaz J, Baccarin T, Mitjans M. Comparative effects of macro-sized aluminum oxide and aluminum oxide nanoparticles on erythrocyte hemolysis: influence of cell source, temperature, and size. J Nanoparticle Res. 2015;17(2):80.