Original research

Hepatotoxicity of naked, serum treated, and plasma treated nanoparticles: an in vitro study

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Abstract

Although metal and metal oxide nanoparticles can be used for medical applications, they have toxicity on different cells such as myocardial cells. The surface properties of nanoparticles can change their cytotoxicity. The purpose of this study was to evaluate the hepatotoxicity of naked, serum treated, and plasma treated of silver nanoparticles (Ag NPs), gold nanoparticles (Au NPs), titanium dioxide nanoparticles (TiO_2 NPs), zinc oxide nanoparticles (ZnO NPs), and magnesium oxide nanoparticles (MgO NPs). To evaluate cytotoxicity, myocyte cells were separately exposed to serial concentrations of nanoparticles for 24 hours at 37 ºC, and then MTT assay, ATP assay, and LDH assay were used. Also, to study toxicity mechanism of nanoparticles, uptake assay and ROS generation were carried out. This study showed that each naked nanoparticle had higher hepatotoxicity than serum treated or plasma treated nanoparticles. Interestingly, both serum treated and plasma treated of each nanoparticle had same hepatotoxicity . Although all naked and coated nanoparticles decreased cell viability and ATP level, and increased the release of LDH enzyme, ROS generation, and uptake, but the highest hepatotoxicity was observed for naked Ag NPs. Also, the minimum toxicity was seen for both serum treated and plasma treated MgO NPs.

Keywords Hepatotoxicity ; Serum treated nanoparticles; Serum treated nanoparticles; Myocyte

Introduction

The use of nanoparticles for heart diseases is a new field, which offers some exciting possibilities including treatment of defective heart valves and myocardial infraction, both detection and treatment of arterial plaque, sub-cellular understanding of heart tissue functions, and imaging of affected myocardial cells [1,2]. Targeted nanoparticles have been used to damage macrophages [3] and blood vessels [4] in the myocardial infraction. Notably, these nanoparticles cross endothelia by enhanced permeation and retention. Previous studies showed the use of targeted nanoparticles for tumor treatment. The feasibility of this procedure has already been shown clinically [5,6]. On the other hand, Annexin-labeled nanoparticles or apoptosis-sensing nanoparticles have been used for detection and imaging of infected heart cells [7]. The use of nanopipet to insert drugs in certain portions of heart cells [8], the use of nanolipoblocker to block LDL and cholesterol molecules [9], and the use of polymer nanoparticles to dissolve inflamed plaque [10] are good examples, which show nanoparticles will improve treatment of heart diseases and imaging of affected myocardial cells in future.

Metal and metal oxide nanoparticles widely have been studied for medical application including implants, tissue engineering, drug delivery, antimicrobial effects, etc [11,12]. Also, these nanoparticles may be applied for detection and treatment of heart diseases. TiO_2 nanoparticles have been used in tissue engineering and in new medical constructs [13]. Also, gold nanoparticles have been applied to detect biomarkers and infectious agents [14], and may be for both imaging and drug delivery in future. Some nanoparticles such as Silver, MgO, and ZnO nanoparticles have antimicrobial property [15-17] and can be coated on surface of new cardiac constructs or integrated in composites. Although nanoparticles have different advantages, their toxicity must be thought. Nanoparticles, which used in medical application, are entered the body via six routes including dermal, subcutaneous, intravenous, oral, inhalation, and intraperitoneal. Then, interact with biological components, *e.g.*, proteins, membranes, and DNA. Afterward, nanoparticles distribute to

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different organs such as heart, liver, spleen, kidney, and brain. They can be modified or metabolized in these organs. Finally, nanoparticles enter in the cells and remain for an unknown time or some of them are excreted [18]. According to previous study, decrease of mitochondrial function and increase of LDH leakage were observed after nanoparticle cell entrance [19]. Previous studies showed that nanoparticles interact with different compounds of cells including membrane, proteins, DNA, and lead to cell damaging or cell killing [20]. The mechanisms of nanoparticle toxicity have not been well known, but catalytic oxidation, binding to active protein and cell component, ion releasing, producing reactive oxygen species (ROS) are some mechanisms. It must be mentioned that toxicity of nanoparticles is related to physical and chemical properties *e.g.*, shape, crystal structure size, porosity, surface charge, area, and functional groups [21]. As soon as nanoparticles enter to human body, they interact with extra cellular fluid (ECF) and then affect heart cells. We propose ECF interacted nanoparticles have different toxic effects vs. non interacted nanoparticles (naked nanoparticles).

Although there are some studies about toxicity of metal and metal oxide nanoparticles [22,23], little information was reported about their toxicity on heart cells. Jawad et al. demonstrated that TiO_2 nanoparticles at concentration of 10 µg/mL had no significant effect on myocyte over 24 hours, but TiO_2 nanoparticles at concentration of 100 µg/mL led to low heart contraction [13]. In 2013, Du et al. worked on cardiovascular toxicity of silica nanoparticles at different sizes and different dosages in Wistar rats, and showed silica nanoparticle transit to alveolar-capillary barrier, and suggested that the nanoparticle uptake and related toxicity were depended on the particles size and dosage [24]. According to another study, gold nanoparticles induced cardiac tissue damage, which depended on the size and time exposure [25].

Because of low data about toxicity of metal and metal oxide nanoparticles on heart cells, the aim of this study was to evaluate toxicity of naked, serum treated, and plasma treated of silver

nanoparticles (Ag NPs), gold nanoparticles (Au NPs), titanium dioxide nanoparticles (TiO_2 NPs), zinc oxide nanoparticles (ZnO NPs), and magnesium oxide nanoparticles (MgO NPs) on myocyte cells of Balb/c mice.

Materials and methods

Materials

Ag NPs, Au NPs, TiO_2 NPs, ZnO NPs, and MgO NPs were provided from Lolitech Company, Germany. RPMI $_{1640}$, nitrilotriacetic acid, collagenase, hyaluronidase, 5-(and-6)-chloromethyl-2', 7'-dichlorodihydrofluorescein diacetate acetyl ester (CM-H2DCFDA), and 3-(4,5-dimethylthiazol-2-yl)-2, 5-diphenyl-tetrazolium bromide (MTT) were purchased from Sigma-Aldrich Chemical Co, (St Louis, MO). Also, Hanks' balanced salt solution (HBSS), and ATP determination reagent (CellTiter-Glo) were obtained from Gibco Invitrogen. LDH kit was from Pars Azmoon Company, Iran and Balb/c mice were provided from Pasteur Institute of Iran. In this research, perfusion solution was Krebs- Henseleit (KH), which included NaCl, KCl, MgSO4, KH_2PO4, NaHCO3, Glucose, $CaCl_2$ at concentration of 119, 4.7, 0.94, 1.2, 25, 11.5, 1 mmol/L, respectively.

Preparation and characterization of naked, serum treated, and plasma treated nanoparticles

Firstly, serial concentration of all nanoparticles (31, 62, 125, 250, and 500 µg/mL) were separately prepared in RPMI $_{1640}$ medium, shacked for 5 minutes, and stored at 5 ºC. In the next step, 10 mL of total blood was obtained from 10 male Balb/c mice. To prepare serum, any anticoagulant was not added, and blood was centrifuged at 1500 rpm after 30 minutes, and its serum was isolated. On the other hand, to prepare plasma, 1 µL of heparin was added to 5 mL of mouse blood, and shacked at room temperature for 10 minutes. Then, heparinized blood was centrifuged at 1500 rpm, and its

plasma was isolated. To prepare serum treated and plasma nanoparticles, one mL of each nanoparticle at concentration of 200 µg/mL was separately added to one mL of mouse serum and mouse plasma, respectively. Then, they were incubated for 1 hour at 37 ºC. After incubation, treated nanoparticles were centrifuged at 10000 rpm, and supernatant was discarded. Then, nanoparticle pellets were resuspended in RPMI $_{1640}$. Same as naked nanoparticles, serial concentrations (31, 62, 125, 250, and 500 µg/mL) of serum treated and plasma treated nanoparticles were prepared in RPMI $_{1640}$. To study chemical composition of naked, serum treated, and plasma treated nanoparticles, Fourier transform infra red spectroscopy (FTIR) (ELICO, India) was used. Also, the size distribution and structure of all type of nanoparticles was studied by dynamic light scattering (DLS) (Malvern Instruments, Italy) and scanning Electron Microscopy (SEM) (Hitachi S-2400), respectively.

Isolation of Balb/c mouse myocytes

Ten male Balb/c mice with weight of 18-20 g were involved in this study. Adult myocytes were isolated by the Langendorff perfusion method [26]. Briefly, the heart of Balb/c mice was perfused by KH at pH 7.4 with 95% O_2 and 5% CO_2 for 5 min, and then perfusion was followed by low calcium solution for 5 minutes. Final perfusion was carried out for 20 minutes by same solution, but with collagenase at 0.6 mg/mL, hyaluronidase at 0.5 mg/mL, calcium at 200 mM, and without nitrilotriacetic acid. Then, left and right ventricles were separated, chopped, and incubated with enzyme solutions (collagenase and hyaluronidase) at 37 ºC for 15 minutes. Isolated cells were passed through 300 µm mesh size filter, and centrifuged at 3000 rpm for 3 minutes. Finally, myocyte cells were washed and resuspended in RPMI $_{1640}$ medium. The final concentration was 1000 myocyte cells/mL. This concentration of cells was used for all experiments

MTT assay

First, myocyte cells were separately incubated with serial concentrations of naked, serum treated, and plasma treated nanoparticles at 37 ºC for 24 hours, and then were washed three times with HBSS. After incubation, 100 µL of RPMI $_{1640}$ and 25 µL of MTT at concentration of 5 mg/mL were added to cells (1000 myocyte cells/ well) in 96-well micro plate, and incubated at 37 ºC for 4 hours. In the final step, 100 µL of isopropanol (70% v/v) was added, and then optical density (OD) of wells was read by a micro plate reader (Novin Gostar, Iran) at 490 nm. Finally, all OD were normalized to control, *i.e.*, the OD of each well was divided to OD of negative control, which was not treated with any nanoparticles.

LDH assay

The quantity of released LDH enzymes was measured by LDH assay kit, according to kit procedure. Firstly, myocyte cells were separately treated with serial concentrations of naked, serum treated, plasma treated nanoparticles at 37 ºC for 24 hours, then cell medium centrifuged at 3000 rpm for 15 minutes, and supernatant was used for LDH assay. Briefly, one mL of reagent 1 (Lactate) and one mL of reagent 2 (NADH) were mixed, and then 10 µL of cell supernatant was added to mixture. The average OD of each sample was read by micro plate reader at 340 nm in 5 minutes. In negative control, cells were not treated with any nanoparticles. In the final step, all data were normalized to control, *i.e.*, the OD of each well was divided to OD of negative control.

ATP assay

The myocyte cells were separately exposed to serial concentrations of naked, serum treated, and plasma treated nanoparticles at 37 ºC for 24 hours, and then cells were washed with HBSS. In the next step, 100 µL of ATP determination reagent and 100 µL of RPMI $_{1640}$ were added to cells, and incubated 10 min at room temperature. Finally, emission intensity of each sample was read by

luminometer (Turner Biosystems, model 9100-102, USA), and normalized to control, *i.e.*, the emission intensity of each sample was divided to emission intensity of negative control. Myocyte cells which were not treated with any nanoparticles considered as negative control.

Mechanism of toxicity

To study mechanisms of hepatotoxicity of nanoparticles, nanoparticle uptake and ROS generation were done.

Uptake assay

First, myocyte cells were treated with naked, serum treated, plasma treated nanoparticles at concentration of 500 µg/mL for 24 hours at 37 ºC, and then cells were washed by HBSS three times. In the next step, cells were treated with 10 M HCl for 12 hours, and concentration of each nanoparticle was quantified by atomic absorption spectrometer (Model 603, Perkins- Elmer, USA).

ROS generation

Briefly, 100 µL of myocyte cells was pretreated with 100 µL of CM-H2DCFDA at concentration 10 µM, and incubated for 1 hour at 37 °C. Then, myocyte cells were exposed to naked, serum treated, plasma treated nanoparticles at concentration of 500 µg/mL for 24 hours at 37 ºC, and then cells were washed by HBSS three times. Then, generated fluorescence was read by a Cytofluor series 4000 plate reader (PerSeptive Biosystems, Inc., Framingham, MA) at 485 nm excitation and 530 nm emission. In the final step, all data were normalized to control, *i.e.*, the fluorescence intensity of each sample was divided to the fluorescence intensity of negative control which was not treated with any nanoparticles.

Statistical analysis

All tests were repeated three times and results were demonstrated as mean ± standard deviation (SD). Then, parametric test (Student's t-test) was applied to evaluate the significant differences. In this study, SPSS software (V.16.0 for Windows; SPSS Inc., Chicago, IL) was used, and $P<0.05$ value was considered as statically significant difference.

Results

Characterization of serum treated nanoparticles

Naked, serum treated nanoparticles, and plasma treated nanoparticles were characterized by SEM and FTIR. Fig. 1 (a, b, c, d, and e) shows SEM images of naked nanoparticles. As shown, agglomeration of all naked nanoparticles was seen in RPMI $_{1640}$ medium. But in case of serum and plasma treated nanoparticles, no agglomeration was observed (Fig. 1f, 1g, 1h, 1i, 1j, 1k, 1l, 1m, 1n, 1o). Clearly, there were same size and structure for serum and plasma treated nanoparticles of each nanoparticle. These images demonstrate round-shape of all coated nanoparticles with no agglomeration. The size distribution of naked, serum treated, and plasma treated nanoparticles was about 100-1000, 25, and 25 nm, respectively. The same distribution size was seen for serum treated and plasma treated nanoparticles. According to FTIR spectrum, serum treated and plasma treated of each nanoparticle had same surface composition, *i.e.*, all serum treated and plasma treated nanoparticles had amide band I (1650 cm^{-1}), amide band II (1550 cm^{-1}), and NH stretching vibrations – amide A and B (3170-3300 cm^{-1}). These spectrums were not seen in naked nanoparticles. It must be mentioned that these spectrums are specific vibration of protein molecules.

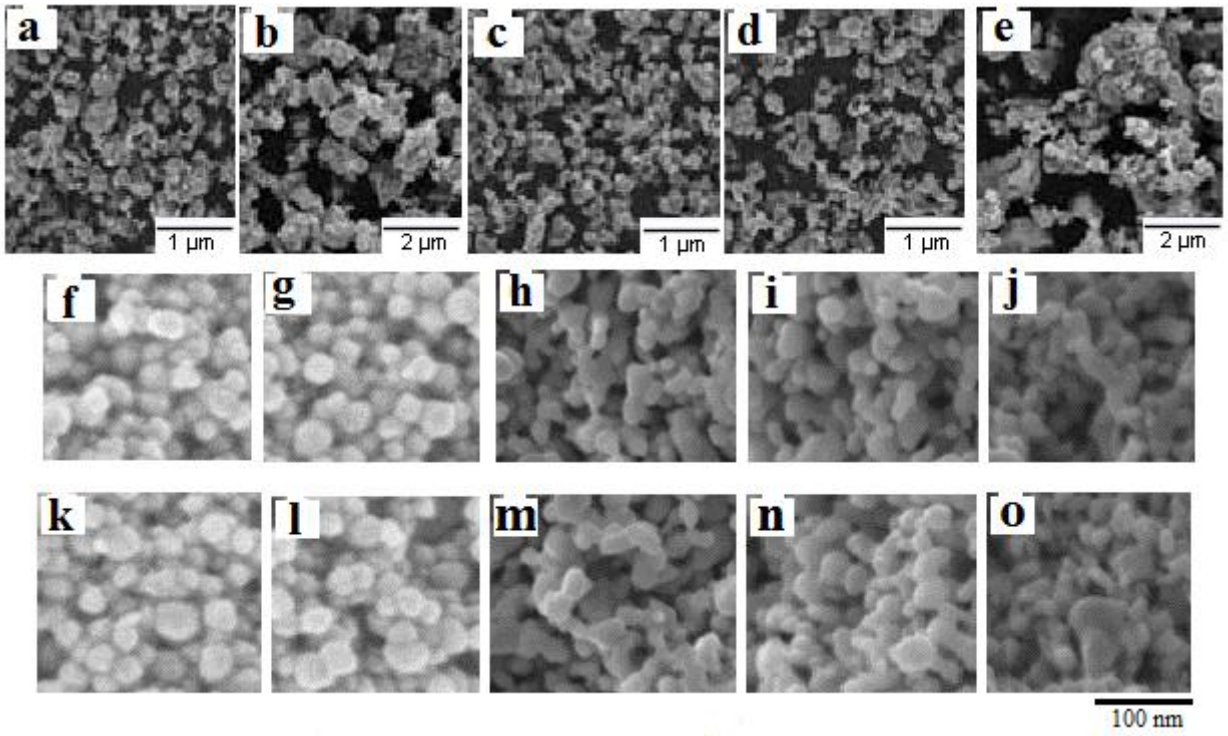

Fig. 1 SEM images of nanoparticles. Naked Ag NPs, Au NPs, TiO_2 NPs, ZnO NPs, and MgO NPs are shown in Fig. 1a, 1b, 1c, 1d, and 1e, respectively. Serum treated Ag NPs, Au NPs, TiO_2 NPs, ZnO NPs, and MgO NPs are demonstrated in Fig. 1f, 1g, 1h, 1i, and 1j. Also, plasma treated Ag NPs, Au NPs, TiO_2 NPs, ZnO NPs, and MgO NPs are seen in Fig. 1k, 1l, 1m, 1n, 1o, respectively. Note: figure from F to O has same scale bar.

Hepatotoxicity results

To evaluate toxicity of naked, serum treated, and plasma treated nanoparticles, three assays were used including MTT assay, LDH assay, and ATP assay. Hepatotoxicity of naked, serum treated, and plasma treated Ag NPs, Au NPs, TiO_2 NPs, ZnO NPs, and MgO NPs are shown in Fig. 2, 3, 4, 5, and 6, respectively. Each figure contains MTT, LDH, and ATP results. All nanoparticles affected myocyte cells, and led to decrease of cell viability and ATP level, and increase of LDH enzymes. As shown in figures, naked form of each nanoparticle leads to less cell viability, less ATP level, and

higher LDH enzymes than serum treated or plasma treated forms ($P<0.05$). In case of each nanoparticle, there were no differences in cell viability, ATP, and LDH level between serum treated and plasma treated forms ($P>0.05$). As seen in these figures, hepatotoxicity of all naked and coated nanoparticles are dose-dependent. It means that high concentration of nanoparticles led to low cell viability, high LDH release, and low ATP level. Although there were no significant differences between hepatotoxicity of different naked nanoparticles ($P>0.05$), the highest toxicity was observed for naked Ag NPs. Au NPs, TiO_2 NPs, ZnO NPs, and MgO NPs were less toxic. Exactly, this order was seen for serum treated and plasma treated nanoparticles. As demonstrated in Fig. 7, the minimum toxicity was observed for serum treated and plasma treated MgO NPs. EC50 for all naked, serum treated, and plasma treated nanoparticles was about 500 µg/mL.

Mechanism of toxicity

To find mechanisms of toxicity of naked, serum treated, and plasma treated nanoparticles, uptake assay and ROS generation assay were used. The results of uptake and ROS generation are shown in fig. 7a and fig. 7b, respectively. These figures show each naked nanoparticle has higher uptake and ROS generation than serum treated and plasma treated nanoparticle ($P<0.05$). On the other hand, the highest uptake and ROS generation is observed for naked Ag NPs, and the least uptake and ROS generation was seen for both serum treated and plasma treated MgO NPs.

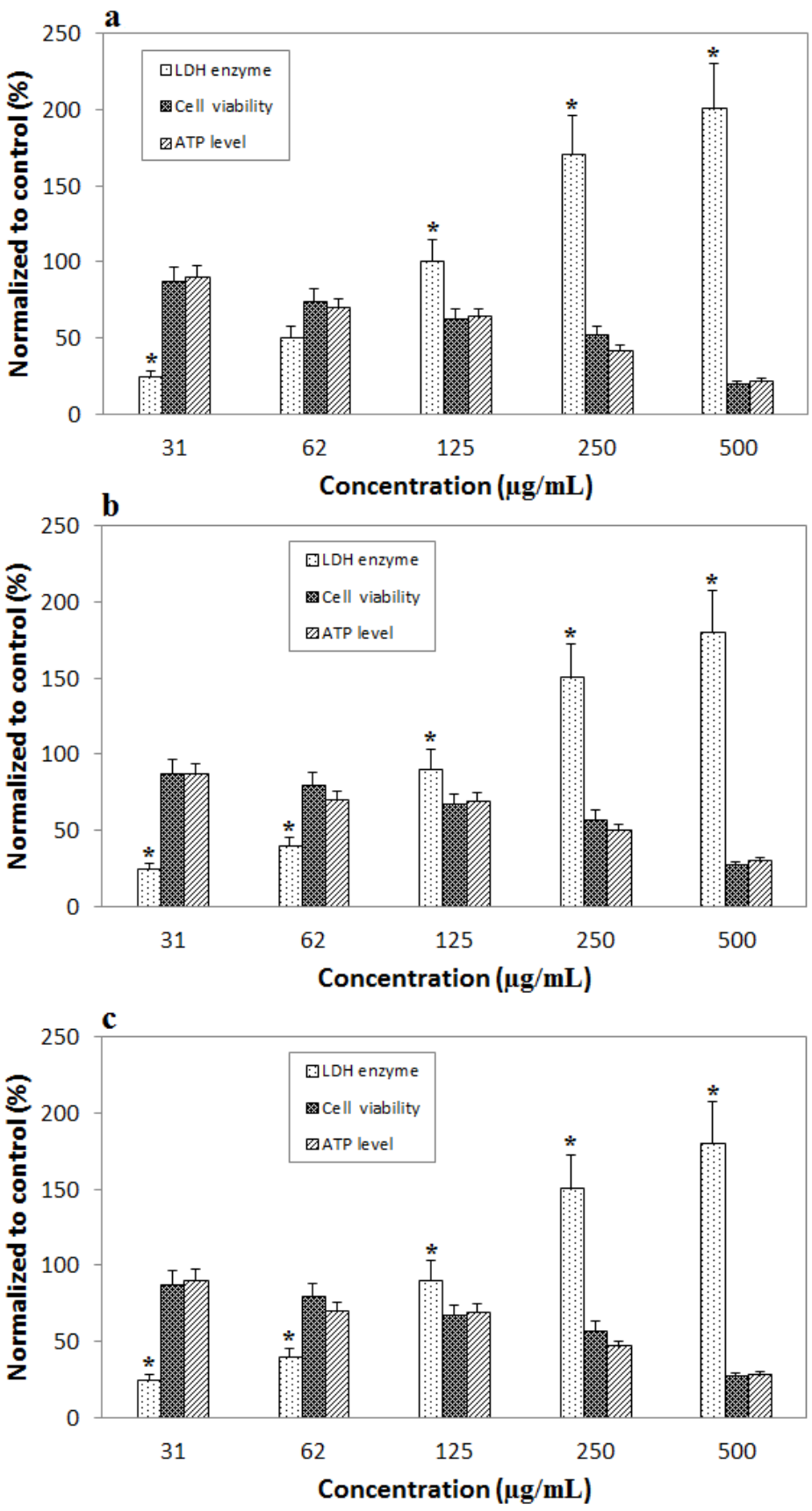

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Fig. 2 The effects of Ag NPs on myocyte cells. First, myocyte cells were separately incubated with serial concentrations of naked (a), serum treated (b), and plasma treated (c) Ag NPs for 24 hours at 37 ºC. Then, cell viability, LDH, and ATP level were measured by MTT assay, LDH kit, and CellTiter-Glo reagent, respectively. All data were shown as mean ± SD with n=10 (independent tests). *P<0.05 compared with quantity of cell viability and ATP level at same concentration.

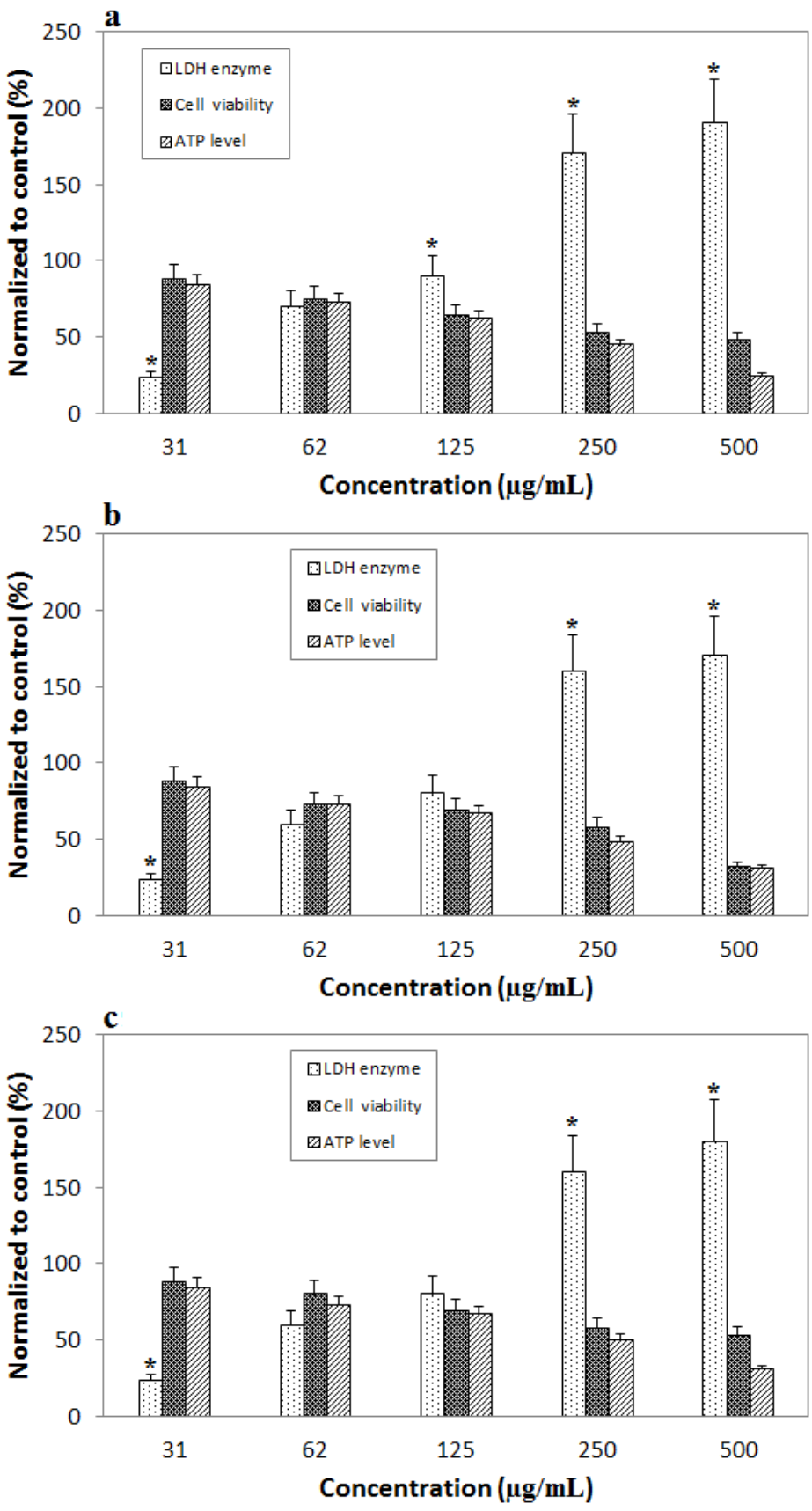

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Fig. 3 The effects of Au NPs on myocyte cells. Myocyte cells were separately incubated with serial concentrations of naked (a), serum treated (b), and plasma treated (c) Au NPs for 24 hours at 37 ºC. Then, cell viability, LDH, and ATP level were measured by MTT assay, LDH kit, and CellTiter-Glo reagent, respectively. All data were shown as mean ± SD with n=10 (independent tests). *P<0.05 compared with quantity of cell viability and ATP level at same concentration.

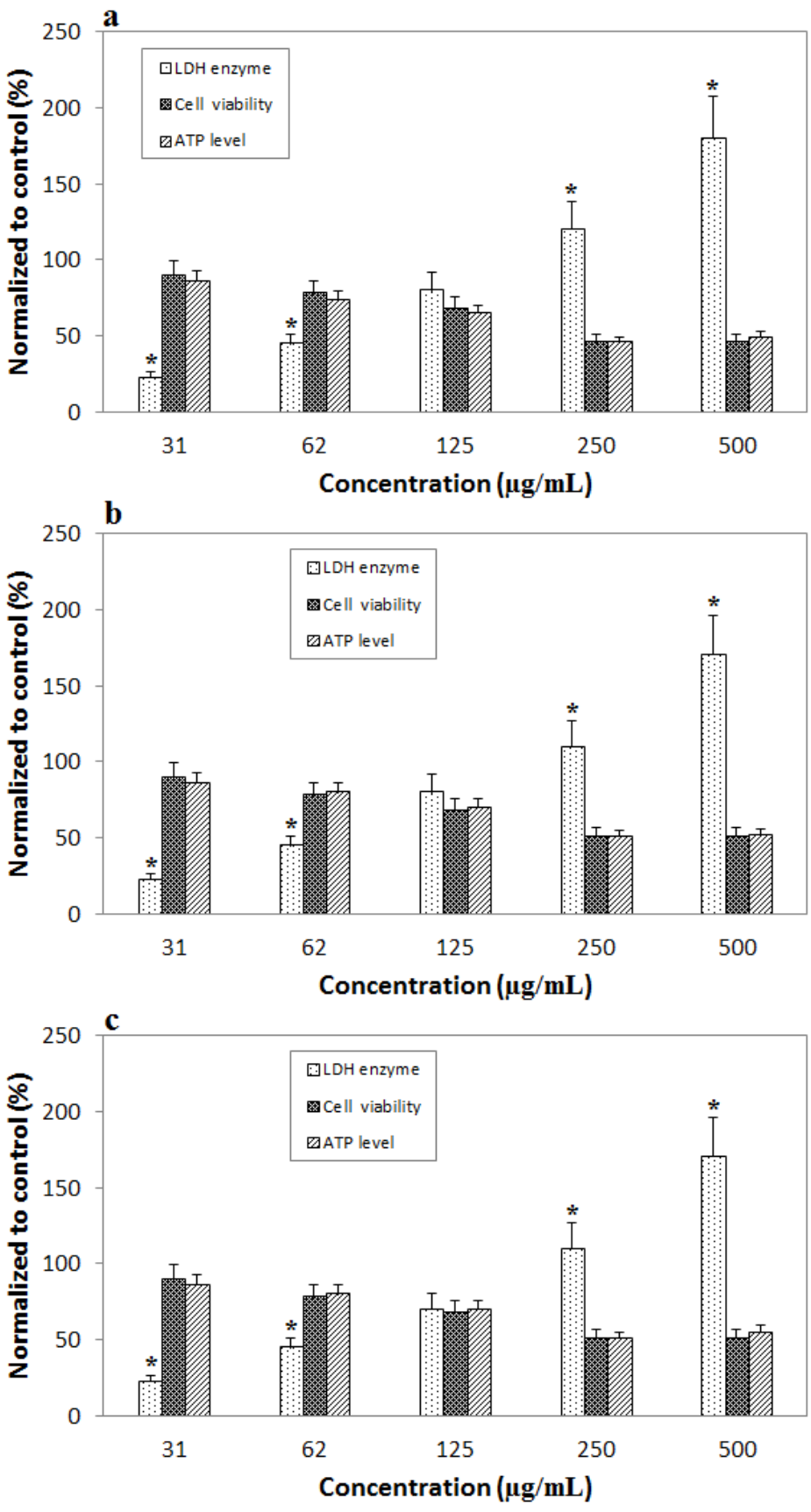

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Fig. 4 The effects of TiO_2 NPs on myocyte cells. First, myocyte cells were separately incubated with serial concentrations of naked (a), serum treated (b), and plasma treated (c) TiO2 NPs for 24 hours at 37 ºC. Then, cell viability, LDH, and ATP level were measured by MTT assay, LDH kit, and CellTiter-Glo reagent, respectively. All data were shown as mean ± SD with n=10 (independent tests). *P<0.05 compared with quantity of cell viability and ATP level at same concentration.

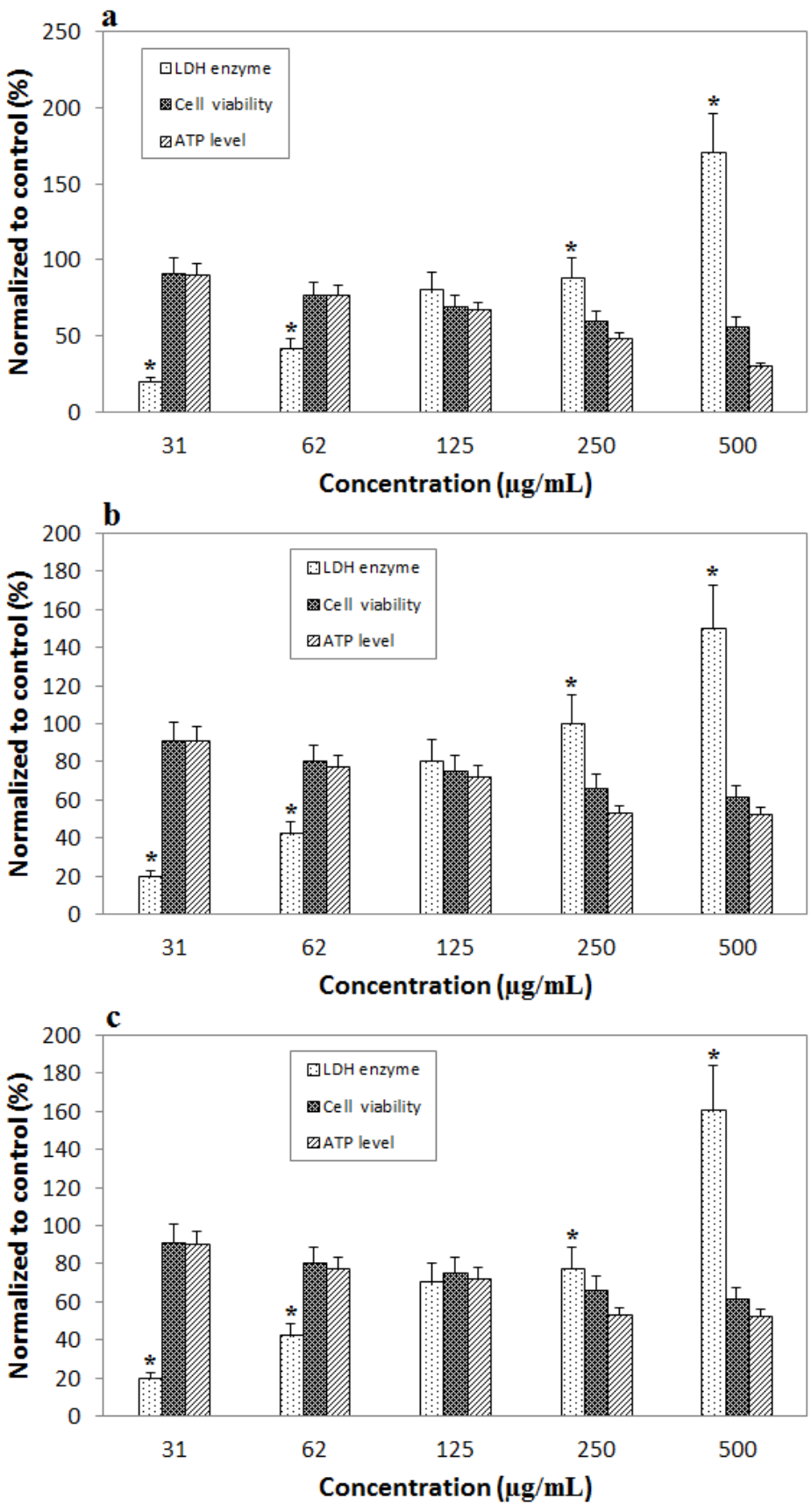

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Fig. 5 The effects of ZnO NPs on myocyte cells. Myocyte cells were separately incubated with serial concentrations of naked (a), serum treated (b), and plasma treated (c) ZnO NPs for 24 hours at 37 ºC. Then, cell viability, LDH, and ATP level were measured by MTT assay, LDH kit, and CellTiter-Glo reagent, respectively. All data were shown as mean ± SD with n=10 (independent tests). *P<0.05 compared with quantity of cell viability and ATP level at same concentration.

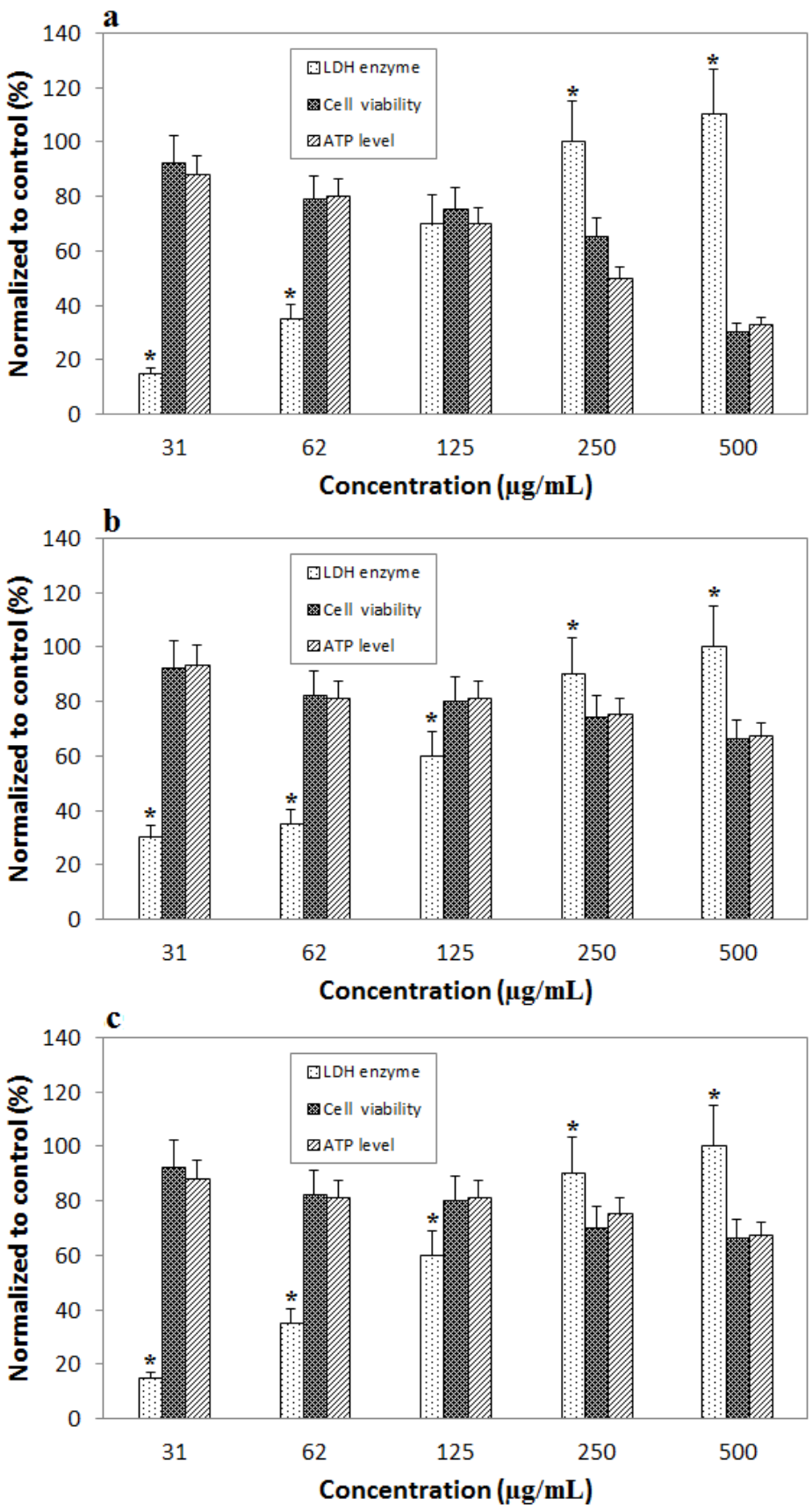

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Fig. 6 The effects of MgO NPs on myocyte cells. Myocyte cells were separately incubated with serial concentrations of naked (a), serum treated (b), and plasma treated (c) MgO NPs for 24 hours at 37 ºC. Then, cell viability, LDH, and ATP level were measured by MTT assay, LDH kit, and CellTiter-Glo reagent, respectively. All data are shown as mean ± SD with n=10 (independent test). *P<0.05 compared with quantity of cell viability and ATP level at same concentration.

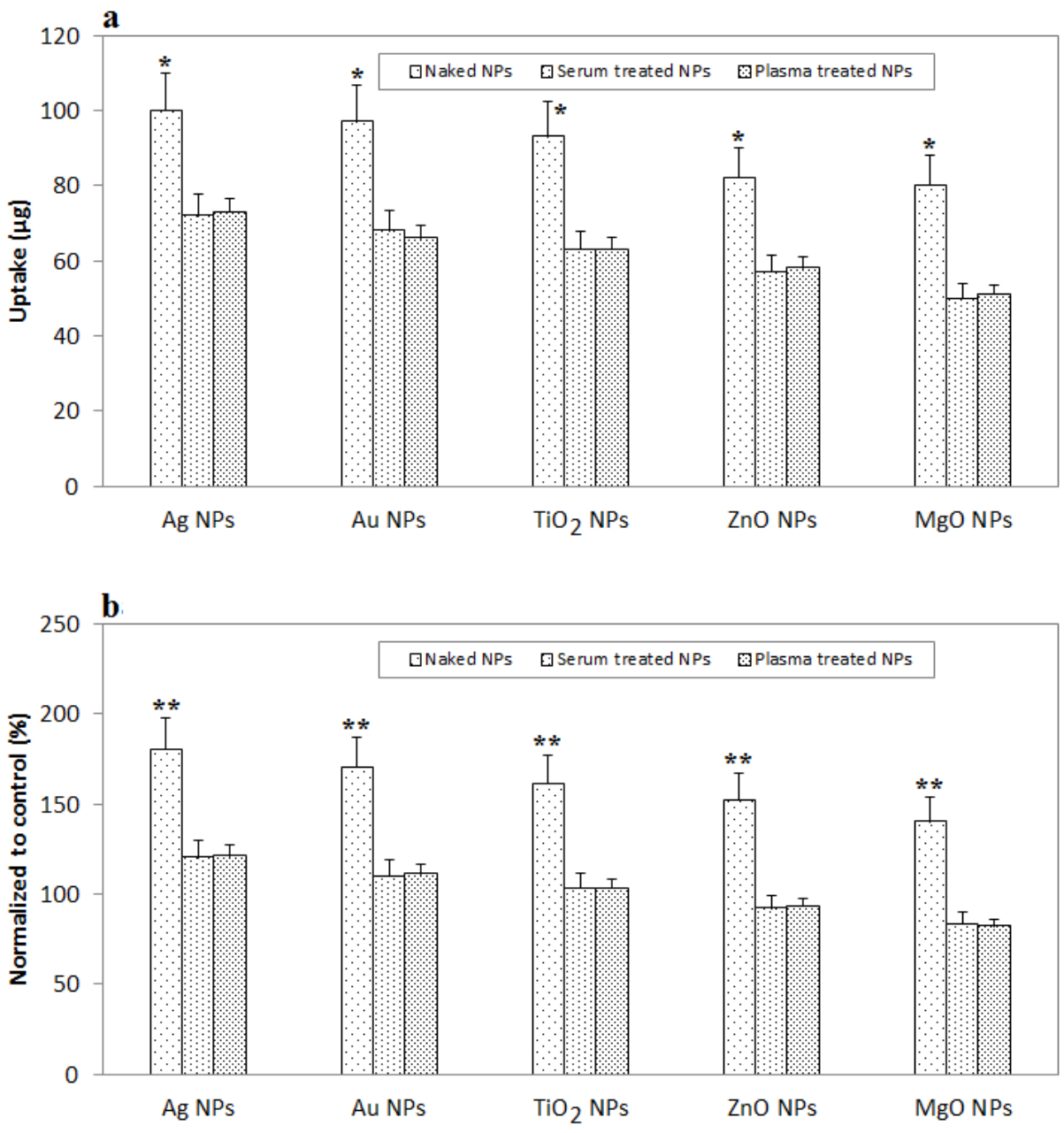

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Fig. 7 Nanoparticle uptake and ROS generation. Myocyte cells were separately incubated with naked, serum treated, and plasma treated nanoparticles at concentration of 500 µg/mL for 24 hours. Then, cells treated with HCl and nanoparticle uptake was measured by atomic absorption spectrometer. The uptake results are shown in Fig. 7a. For ROS generation assay, generated fluorescence was read at 485 nm excitation and 530 nm emission. ROS generation results (Fig. 7b) were normalized to control, and were shown as mean ± SD with n=10 (independent test). *P<0.05 compared with the uptake of serum treated and plasma treated form of same nanoparticle. **P<0.05 compared with the ROS generation of serum treated and plasma treated form of same nanoparticle.

Discussion

Because of chemical and physical properties of metal and metal oxide nanoparticles, they are good candidate for wide medical application including tissue engineering, coating on the implant, drug delivery, etc [11,12]. In case of cardiology, nanoparticles can be used for imaging of affected myocardial cells, treatment of heart diseases, and studying of molecular functions of heart cells [1,2]. In spite of nanoparticle advantages, they have cytotoxicity which dependent to size, shape, and surface properties [22,23]. There was little information on effect of metal and metal oxide nanoparticles on heart cells. Thus, the purpose of this study was to evaluate hepatotoxicity of naked, serum treated, and plasma treated Ag NPs, Au NPs, TiO_2 NPs, ZnO NPs, and MgO NPs.

This study showed that naked form of each nanoparticle led to less cell viability, less ATP level, higher LDH enzyme release, higher uptake, and higher ROS generation than serum treated and plasma treated forms. The higher toxicity of naked nanoparticles than serum coated nanoparticles has been reported in other studies [27]. The reason of this pattern is because of protein adsorption of nanoparticles. The layer of proteins on the surface of nanoparticles inhibits direct interaction between nanoparticles and cell compounds such as different membranes, enzymes, and DNA. This

fact leads to higher toxicity of naked nanoparticles than serum treated nanoparticles. On the other hand, naked nanoparticles aggregate in RPMI $_{1640}$, because of electrostatic forces. Authors hypothesized that the formation of micron size of metal and metal oxide in and out of cell is responsible of higher cell death than coated nanoparticles. This research clearly showed that there was no difference between toxicity of serum treated and plasma treated forms of each nanoparticle ($P>0.05$). The authors hypothesize that since the composition of both serum and plasma is approximately similar, the same pattern of toxicity was seen for both serum and plasma coated nanoparticles. Exactly, the difference of serum and plasma is in clothing factors such as fibrinogen, but these factors did not change hepatotoxicity .

In case of naked nanoparticles, the highest toxicity was seen for naked Ag NPs, and the least toxicity was observed for MgO NPs. The order of hepatotoxicity was as following: Ag NPs>Au NPs>TiO_2 NPs>ZnO NPs>MgO NPs. A significant difference was only seen between hepatotoxicity of Ag NPs and MgO NPs ($P<0.05$), but there were no significant differences between other nanoparticles ($P>0.05$). Interestingly, this order was observed for serum treated and plasma treated nanoparticles. The authors explain that the reason of high toxicity of Ag NPs is due to high uptake and high ROS generation, according to Fig. 7a and Fig. 7b. The higher uptake and ROS generation leads to higher damage of cell compounds, inhibition of enzymes, and cell death. Other mechanisms such as high ion release and oxidation property may be included, which must be further in future study. In consistent with other studies, previous studies showed that toxicity of metal and metal oxide nanoparticles on different cells [23,22]. As noted, nanoparticles interact with proteins, DNA, and cell membranes, and some reports showed that some nanoparticles lead to DNA damage, caspase activation, condensation of chromatin, formation of micronuclei, and lipid peroxidation [20,21]. Another finding was direct relationship between nanoparticle uptake and ROS

generation vs. LDH quantity, and reverse relationship between nanoparticle uptake and ROS generation vs. cell viability and ATP level, which was in agreement with previous studies [28].

Nanoparticles can affect myocardial cells by injury of epithelial tissue [29], oxidative stress response, and inflammation [30,31]. Some researchers have also indicated oxidative stress response of nanoparticles is an important mechanism of toxicity [32]. Mann et al. proposed that oxidative stress, inflammation, and autonomic dysregulation are some proposed mechanisms of toxic effect of nanoparticles on heart cells. On the other hand, they declared that nanoparticles can change vascular endothelial cell integrity, and lead to disruption of heart rate, electrical activity, and increase susceptibility to ischemia/reperfusion injury [33]. There are no data about serum treated and plasma treated nanoparticles on heart tissue, but some studies have been reported on naked nanoparticles on heart cells, which discussed here. Abdelhalim et al. showed that exposure to Au NPs produce cardiac damages, depended on the size and total exposure. According to their results, Au NPs with size of 10 and 20 nm were more toxic than size of 50 nm. Also, 7 days administration of nanoparticles was more effective than 3 days administration [25]. Interestingly, as seen in other study, PEGylated Au NPs can affect cardiovascular function [34]. Jawad et al. showed that TiO_2 NPs at concentration of 10 µg/mL for 24 hours exposure had no effect on rat myocyte, but concentration of 100 µg/mL led to reduction of contraction amplitude [13]. Also, TiO_2 NPs reduced fibroblast proliferation and cell viability at concentration of 5–150 µg/mL after 4 days exposure. Mallik et al. showed that Pt-TiO_2 NPs had great uptake in cardiac cells, and caused increase of oxidative stress and decrease of mitochondrial membrane potential. They suggested Pt modification of TiO_2 NPs led to more cardiac toxicity [35]. Meanwhile, other metal oxide nanoparticles such as silica nanoparticles are toxic to myocardial tissue. According to Du et al. study, silica nanoparticles could pass through the alveolar-capillary and reach to heart, and its uptake is depended on nanoparticle dosage and size [24]. This nanoparticle increases interleukin-1beta, interleukin-6,

tumor necrosis factor-alpha, intercellular adhesion molecule-l, vascular cell adhesion molecule-l. Stampfl et al. suggested Langendorff heart as a model for nanoparticle investigation, which enables observation and analysis of heart parameter over 24 hours. Their study showed that TiO_2 and SiO_2 increased heart rate and arrhythmia [26]. On the other hand, some nanoparticles are cardio protective. Niu et al. demonstrated that cerium oxide nanoparticles protect heart against the progression of cardiac dysfunction by their antioxidant properties [36]. In order to further finding, the authors suggest that other tests such as tissue antioxidant enzymes, lipid peroxidation, cytokine production, and ultra-structural changes must be evaluated in future.

Conclusions

This study declared that each naked nanoparticle had higher toxicity on myocyte cells than serum treated or plasma treated nanoparticles. Also, same hepatotoxicity was observed for both serum and plasma treated of each nanoparticle. Between all naked nanoparticles, Ag NPs had the highest hepatotoxicity with highest uptake and ROS generation, and MgO NPs had the least toxicity. Exactly, this pattern was seen in serum and plasma coated nanoparticles.

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