The effect of novel magnetic nanoparticles on vascular endothelial cell function in vitro and in vivo

Le Su a, Lei Han a, Fei Ge b, Shang Li Zhang a, Yun Zhang c, Bao Xiang Zhao b, Jing Zhao a, Jun Ying Miao a,c,*

a Shandong Provincial Key Laboratory of Animal Cells and Developmental Biology, School of Life Science, Shandong University, Jinan 250100, China
b Institute of Organic Chemistry, School of Chemistry and Chemical Engineering, Shandong University, Jinan 250100, China
c The Key Laboratory of Cardiovascular Remodeling and Function Research, Chinese Ministry of Education and Chinese Ministry of Health, Shandong University Qila Hospital, Jinan 250100, China

HIGHLIGHTS

► The novel nanoparticles could internalize in HUVECs and diffuse in the cytoplasm.
► 1–200 µg/ml MNPs did not affect HUVECs and could be safe to HUVECs in vitro.
► 400 µg/ml MNPs inhibited cell growth regulated by caveolin-1 and eNOS.
► 20 mg/kg MNPs damaged endothelium in the aortic root after injected for 3 days.
► After injected for 6 days and 9 days, the endothelium recovered the integrity.

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ABSTRACT

Manufactured nanoparticles are currently used for many fields. However, their potential toxicity provides a growing concern for human health. In our previous study, we prepared novel magnetic nanoparticles (MNPs), which could effectively remove heavy metal ions and cationic dyes from aqueous solution. To understand its biocompatibility, we investigated the effect of the nanoparticles on the function of vascular endothelial cells. The results showed that the nanoparticles were taken up by human umbilical vein endothelial cells (HUVECs) and could inhibit cell proliferation at 400 µg/ml. An increase in nitric oxide (NO) production and endothelial nitric oxide synthase (eNOS) activity were induced, which accompanied with the decrease in caveolin-1 level. The endothelium in the aortic root was damaged and the NO level in serum was elevated after treated mice with 20 mg/kg nanoparticles for 3 days, but it was integrated after treated with 5 mg/kg nanoparticles. Meanwhile, an increase in eNOS activity and decrease in caveolin-1 level were induced in the endothelium. The data suggested that the low concentration of nanoparticles could not affect the function and viability of VECs. The high concentration of nanoparticles could inhibit VEC proliferation through elevation of the eNOS activity and NO production and thus present toxicity.

1. Introduction

Nanotechnology is a fast growing industry with seemingly limitless applications in many fields such as cosmetics and skin care products, drug delivery devices, diagnosis, controlling of biological systems and environmental pollution [1,2]. Manufactured
nanoparticles created in the diameter range between 1 and 100 nm, display unique physicochemical characteristics due in part to their smaller size, large surface-to-volume ratio, and increased reactivity [3]. As early as 2006, it was predicted that 58,000 metric ton nanoparticles were produced per year until 2011 [4]. Because of the diverse potential of nanoparticles, it is estimated that the occupational and public exposure of manufactured nanoparticles will dramatically increase in the future. With more and more applications of nanomaterials, it is found that they have negative health and environmental impacts [5]. However, it is lack of available toxicity data nowadays.

Increasing reports indicate that exposure to ultrafine particulate matter (diameter <100 nm) is associated with increases in cardiovascular morbidity and mortality [6]. Recently, people pay much attention to the correlation between exposure to nanoparticles and cardiovascular disease [7]. Among the hypotheses on the mechanisms of nanoparticle-induced cardiovascular diseases, most were induced by endothelial dysfunction [3]. Endothelial cells, which line the inner surface of blood vessels, direct contact with the particles, making nanoparticles–endothelial interactions potentially pathogenically relevant [8]. Several studies have shown that intravenously administrated iron nanoparticles can translocate from the blood circulation into various targeted tissues and organs [9]. Furthermore, a large number of studies have demonstrated that nanoparticles in the respiratory tract can also cross the alveolar–capillary barrier into the blood circulatory system of humans and animals [10]. Nanoparticle-triggered endothelial dysfunction is now hypothesized to be a dominant mechanism in the development of the diseases. It is an important regulator providing physical and biological protection of vascular function and homeostasis by secreting many kinds of growth factors [11,12].

It has been reported that a number of nanoparticles have directly effect in the endothelial system, including the growth inhibition of endothelial cells, attenuation of endothelium-dependent vasodilation and increase in the expression of endothelial cell adhesion molecules [3,13,14]. And the biological behavior of nanoparticles and the cytotoxicity induced by nanoparticles have attracted much more concern [15]. However, the exact mechanisms of dysfunction of endothelial system induced by nanoparticles are not yet well known. Thus, detailed and in-depth investigations dealing with this issue are needed.

In our previous study, we prepared novel Fe3O4 magnetic nanoparticles (Fe3O4@APS@AA-co-CA, MNPs) which could remove heavy metal ions and cationic dyes from aqueous solution [16,17]. In the current study, the biological effects of the MNPs exposure on human umbilical vein endothelial cells (HUVECs) and the endothelium in mice were further investigated. The high concentration of MNPs could inhibit the endothelial cell proliferation and induce endothelial cell dysfunction. The possible mechanism was the elevation of nitric oxide (NO) production which was induced by the decrease of caveolin-1 and elevation of endothelial nitric oxide synthase (eNOS) activity.

2. Experimental

2.1. In vitro studies

2.1.1. Cell cultures

HUVECs were obtained in our laboratory by using the method of Jaffe et al. [18]. The cells were cultured as described previously [19]. All experiments were performed on the cells from 10 to 20 passages. The morphological changes were observed under the phase contrast microscope (Nikon, Japan).

2.1.2. Exposure to MNPs

The MNPs is prepared as previously [16]. Briefly, 0.200 g AA-co-CA was immobilized on 0.100 g Fe3O4@APS with catalysis of 0.200 g DCC. The mixture was dispersed in 20 ml distilled THF under ultrasonator for 5 min with mechanical agitation for 3.5 h. The product was separated by magnetic fields and washed with 150 ml anhydrous ethanol, then dried under vacuum at 60 °C. The diameters of the MNPs are typically 15–20 nm. The MNPs possessed superparamagnetic properties.

Thermo-gravimetric properties have shown that high temperature has no significant influence on the structure of MNPs [16]. Besides, the chemical structure of the MNPs is reactive type, which means that the combination is through chemical bonds. As a result, neutral high temperature steam is also supposed not to breakdown the structure. Thus, for experiments in vitro, MNPs were sterilized at 121 °C for 30 min and then were suspended in M199 (Intrivogen, CA, USA) plus 20% fetal bovine serum. Stock solutions were sonicated for 5 min in order to achieve good suspension before they were diluted by complete M199 culture medium to 1, 10, 100, 200, and 400 μg/mL. For experiments in vivo, sterilized MNPs were dispersed in PBS (pH 7.4) with 0.1% Tween 80. The suspensions were sonicated for 5 min before tail vein injection.

2.1.3. Cell viability analysis

Cell viability was determined by WST-8 assay using the cell counting Kit-8 (Sigma Chemical Co., USA, 96992). Cells were seeded into 96-well plates. After cells were treated with MNPs for 44 h, 10 μl of WST-8 solution was added and then the plate was further incubated for 4 h. The aggregated particles were removed by centrifugation at 1500 rpm for 10 min. The percentage of living cells was calculated by the ratio of OD measured at 450 nm.

2.1.4. Cell death assay

Lactate dehydrogenase (LDH) assay was performed on cells treated with MNPs for 48 h using a LDH kit (Nanjing Jiancheng Company, China) according to the manufacturer’s protocol. Light absorption was measured at 340 nm using a model Cintra 5 UV-Vis spectrometer (GBC, Australia).

Cells were stained with 5 μg/ml acridine orange (AO, Fluka) for 5 min at room temperature. The nuclear condensation and fragmentation were observed with a laser scanning confocal microscopy (LSCM). Cells were fixed in 4% formaldehyde for 10 min, then incubated with Hoechst 33342 (2 μg/ml) (Sigma, St. Louis, MO, USA) for staining for 60 min at 37 °C. Stained cells were washed with PBS twice, and then viewed under an Olympus inverted fluorescence microscope. Cells were scored as apoptotic if their nuclei were much brighter or exhibited condensation of chromatin and nuclear fragmentation.

Apoptosis was demonstrated using the Guava Nexin kit and the Guava PCA system (Guava Technologies, Hayward, CA). It utilizes two stains (Annexin V and 7- amino actinomycin D [7-AAD]) to quantify the percentage of apoptotic cells. Cells were harvested after treatment with MNPs for 48 h and diluted to 5 x 10^5 cells/ml as per manufacturer’s recommendations. The Guava Nexin kit reagent was added to the cells and incubated on ice for 20 min prior to analysis on the Guava PCA system. Results are expressed as the percentage of gated cells that are positive for Annexin V staining.

2.1.5. Transmission electron microscopy (TEM)

HUVECs were incubated with MNPs (100 μg/ml) for 48 h in culture dishes (1 x 10^6 cells/dish). Then cells were fixed in 2.5% glutaraldehyde in 0.1 M sodium cacodylate buffer (pH 7.4) and rinsed. Cells were fixed for 1 h in 2% osmium tetroxide with 3% potassium ferrocyanide and rinsed. They were treated with en bloc staining with a 2% aqueous uranyl acetate solution and dehydrated...
through a graded series of alcohol. They were then put into propylene oxide, a series of propylene/Epon dilutions, and embedded. The thin (70 nm) sections were cut on a Leica UC6 ultramicrotome, and images were taken on a JEOL 1200 EX (JEOL, Ltd., Tokyo, Japan) using an AMT 2k digital camera.

2.2.1. Alexa 488-Ac-LDL uptake assay

HUVECs were seeded on 24-well culture plates and treated with MNPs for 48 h. Then, cells were incubated in 10 μg/ml Alexa Fluor 488-acetylated low-density lipoprotein (Alexa 488-Ac-LDL) (Invitrogen, USA) at 37 °C for 4 h. The media was removed and cells were washed once with the culture media. The cells that could take up Alexa 488-Ac-LDL showed red fluorescence with LSCM excitation at 488 nm.

2.2.7. Immunofluorescence assay

Immunofluorescence assay was performed as described [20]. After adding the primary antibodies (rabbit anti-human eNOS, p-eNOS [Ser1177] or caveolin-1 IgG) and appropriate secondary antibodies (fluorescein isothiocyanate [FITC]-goat anti-rabbit IgG; all Biotechnology, Santa Cruz, CA), the samples were evaluated by LSCM (Leica, Germany). Cell nuclei were counterstained with propidium iodide (10 μg/ml) for 15 min at room temperature. Targeted proteins displayed green fluorescence after excitation at 488 nm. Cell nuclei displayed red fluorescence after excitation at 633 nm.

We randomly selected the region of interest and then zoomed in the same frame. The relative fluorescent intensity per cell was the total value of the sample in the zoom scan divided by the total number of cells (at least 200 cells) in the same scan.

2.2.8. NO generation detection

The levels of intracellular NO were detected in cells using a fluorescent probe, 3-amino-4-aminomethyl-2′,7′-difuorescein, diacetate (DAF-FM DA, Beyotime, China) [21]. After treated with MNPs, cells were washed three times with Hanks balanced salt solution (HBSS) solution (pH 7.4). After loading with 5 mM DAF-FM DA at 37 °C for 20 min, cells were rinsed three times with HBSS and maintained in HBSS throughout the experiments. NO production was measured using the LSCM.

The levels of extracellular NO including that in the cell supernatant and serum were detected by NO Colorimetric Assay Kit (Abcam, ab65328) according to the manufacturer’s instructions. The light absorption was measured at 540 nm with use of the SpectraMAX 190 microplate spectrophotometer (GMI Co., USA). Data are expressed relative to standard curve for NO.

2.2.2. Blood and tissue collection

After injected with MNPs or PBS for 3, 6, or 9 days, the mice were killed. Blood was collected from the inferior vena cava. Serum was prepared via centrifugation at 3000 × g for 20 min at 4 °C and stored at −80 °C for NO analysis. The hearts were rapidly removed after perfusion with ice-cold PBS. The adventitia was thoroughly stripped, and the heart, including the aortic root, was snap-frozen in optimal cutting temperature (OCT) embedding medium (Tissue-Tek) for immunohistochemical staining. The animal experimental protocol complied with the Animal Management Rules of the Chinese Ministry of Health (document no. 55, 2001) and was approved by the Animal Care Committee of Shandong University.

2.2.3. Immunohistochemistry assay

Aortic roots were dissected from the animals and immersed in OCT embedding medium. Serial 7-μm-thick cryosections were collected every three sections and mounted on poly-D-lysine-coated slides. Twenty to forty sections were collected for each mouse. The expression levels of CD31 in endothelium of aortic root were analyzed by immunohistochemical SABC staining method. Frozen sections were fixed in 10% buffered formalin for 30 min. After washing with distilled water, blocking serum was applied for 20 min. The sections were incubated with an anti-CD31 monoclonal antibody (1:200) (Santa Cruz Biotechnology) overnight at 4 °C. Negative control sections were incubated with PBS. After washing in PBS, a biotin-marked secondary antibody was applied for 20 min. After washing in PBS, SABC was added for 20 min and then washed with adequate PBS. The slides were viewed under an Olympus inverted fluorescence microscope.

The remaining sections were used for immunofluorescence analysis with the following antibodies: anti-mouse monoclonal eNOS; anti-mouse monoclonal p-eNOS; anti-mouse monoclonal caveolin-1; and anti-mouse monoclonal CD31 (all Santa Cruz Biotechnology, Santa Cruz, CA). After incubation with the appropriate FITC- or TRITC-conjugated secondary antibody, tissue was observed by confocal laser scanning microscopy. We randomly selected the region of interest and then zoomed in the same frame and obtained relative fluorescent intensity per area in the zoom scan.

2.3. Statistical analyses

Data are expressed as mean ± SE. SPSS 11.5 (SPSS Inc., Chicago, IL) was used for statistical analysis. Data were analyzed by one-way ANOVA (followed by Scheffé F test for post hoc analysis). A P < 0.05 was considered statistically significant.

3. Results

3.1. Cell morphology and viability

When HUVECs were exposed to different concentrations of MNPs (1–400 μg/ml), there were no obvious morphological changes in cells of the treated groups and control groups at 24 or 48 h. However, the number of cells was reduced significantly in the groups treated with 400 μg/ml MNPs (Fig. 1A). Then we examined the viability of cells after treated with MNPs. The results showed that MNPs could remarkably decrease the cell viability at high concentrations (400 μg/ml or 800 μg/ml), which was consistent with the results from Fig. 1A and B.
3.2. MNP did not induce cell necrosis or apoptosis

In order to evaluate whether MNP induce or apoptosis in vitro, we firstly determined the release of the cytosolic enzyme LDH into the cell culture medium. The results showed that there was no difference in LDH release from HUVECs between the normal group and the groups treated with MNP for 48 h from 1 μg/ml to 800 μg/ml (Fig. 1C). Then, we examined apoptosis by using acridine orange (AO) and Hoechst 33258 staining. MNP up to 400 μg/ml did not trigger nuclear condensation and fragmentation (Fig. 1D). Additionally, the results from the Annexin V and 7-AAD staining showed that MNP up to 400 μg/ml did not increase the number of apoptotic cells (Fig. 1E). These data suggested that low concentrations of MNP (1–200 μg/ml) did not affect HUVEC growth. However, MNP at high concentration (400 μg/ml) decreased HUVEC viability through inhibiting cell proliferation, but not inducing cell necrosis or apoptosis.
Fig. 2. Subcellular localization of MNPs. Confocal microscopy and transmission electron microscopy were performed on HUVEC exposed to MNPs (100 μg/ml) for 48 h, scale bar: 16 μm. (A–C) Normal HUVECs taken by confocal microscopy. (A) Cell nucleus stained with PI; (B) photograph from the transmission microscopy corresponding to (A). (C) The merge of (A and B). (D) Normal HUVECs taken by transmission electron microscopy (TEM). (E–G) HUVECs treated with MNPs (100 μg/ml) for 48 h. (E) Cell nucleus stained with PI. (F) Photograph from the transmission microscopy corresponding to (E–G) the merge of (E and F). (H) HUVECs treated with MNPs taken by TEM. Arrows showed the MNPs did not enter the cell nucleus.

Fig. 3. The high concentration of MNPs induced HUVEC dysfunction. (A) Uptake of Alexa 488-Ac-LDL by HUVECs and fluorescent intensity of eNOS and p-eNOS in HUVECs treated with different concentrations of MNPs for 48 h, scale bar: 40 μm. (B) Quantification of relative fluorescence intensity per cell of Alexa 488-Ac-LDL uptake. (C) Quantification of relative fluorescence intensity per cell of p-eNOS/eNOS (**P < 0.01 vs. normal, n = 3).
3.3. Cellular localization of internalized MNPs

We initially examined the interactions between HUVECs and MNPs by confocal microscopy. This analysis clearly showed that MNPs could be internalized in HUVEC. Most of the particles localized within the cytoplasm, but did not enter the cell nucleus. Transmission electron microscopy (TEM) showed that the MNPs were aggregated in the cytoplasm and trapped in single membrane

Fig. 4. The high concentration of MNPs increased the NO production. (A) The relative intensity of NO in HUVECs detected by LSCM, scale bar: 40 μm. (B) Quantification of relative fluorescence intensity per cell of NO. (C) The extracellular NO production detected in the cell supernatant (**P < 0.01 vs. normal, n = 3).

Fig. 5. The high concentration of MNPs decreased the caveolin-1 level. (A) The relative intensity of caveolin-1 in HUVECs detected by LSCM, scale bar: 8 μm. (B) Quantification of relative fluorescence intensity per cell of caveolin-1 (**P < 0.01 vs. normal, n = 3).
3.4. The MNPs at high concentration induced HUVEC dysfunction

Since VECs are functionally defined by their capacity to take up acetylated low-density lipoprotein from plasma [22], we found that MNPs at 400 μg/ml decreased the ability of HUVECs to take up Alexa 488–Ac-LDL (Fig. 3A and B). Phosphorylation of endothelial nitric oxide synthase (eNOS) at Ser1177 would increase the activity of eNOS to produce NO [23]. Our results showed that the level of phosphorylated eNOS (p-eNOS) was elevated in the cells exposed to 400 μg/ml MNPs (Fig. 3A and C). The data suggested that 400 μg/ml MNPs could induce HUVEC dysfunction and increase the activity of eNOS.

3.5. The MNPs at high concentration increased NO production

Since 400 μg/ml MNPs could increase the activity of eNOS, we investigated both the intracellular NO level and the extracellular NO production. The results showed that only 400 μg/ml MNPs could increase the intracellular (Fig. 4A and B) and extracellular (Fig. 4C) NO production, which was consistent with the elevation of eNOS activity.

3.6. The level of caveolin-1 was decreased by treatment with 400 μg/ml MNPs

We then examined the level of caveolin-1. The results showed that only 400 μg/ml MNPs could decrease the level of caveolin-1 (Fig. 5), which was corresponding to the changes of eNOS activity and NO production.

3.7. The biocompatibility of MNPs in vivo

To further evaluate the biocompatibility of MNPs in vivo, we injected MNPs into mice via the tail vein (Fig. 6A). Animal weight was monitored during the course of the experiments. The graph presented in Fig. 6B illustrates the fact that MNPs did not alter the general health of the mice. In addition, signs of distress, such as loss of hair or behavioral changes were not observed. The appearances and structures of heart, liver, spleen, lung, kidney and brain did not appear to be modified in MNP-injected mice (data not shown). The serum analysis showed that NO level was elevated after injected with HD-MNPs for 3 days. However, NO production then reverted to the normal level after injection with MNPs for 6 days or 9 days (Fig. 6C).

Next, we investigated the integrity of endothelium by immunohistochemistry assay. The results showed that the endothelium was damaged at the aortic root only after injected with HD-MNPs for 3 days (Fig. 7 arrows). Then, the endothelium reverted to their integrity after injection with MNPs for 6 days or 9 days, which was consistent with the changes of NO level in serum (Fig. 7).

Finally, we examined the activity of endothelial NOS and the caveolin-1 level in the endothelium of the aortic roots. Because the caveolin-1 was not only expressed in endothelial cells, we used CD31 to show the endothelium. As shown in Fig. 8, the increased activity of eNOS and the decreased caveolin-1 level were found in the aortic root only after injected with HD-MNPs for 3 days. Then, they reverted to their normal levels after injection with MNPs for 6 days or 9 days, which was consistent with the changes of NO level in serum and the results in vitro.

4. Discussion

Endothelium is the body’s first line of defense for nanomaterials. In this study, to contact the blood vessel directly, we selected tail vein injection. And the cultured human umbilical vein endothelial cells (HUVECs) were applied as the in vitro model of the endothelial monolayer in vessels. The endothelial semi-permeable barrier controls the transfer of many soluble and insoluble substances via two pathways: transcellular and paracellular pathways [24]. The transcellular pathway transports substances via transcytosis in vesicle carriers whereas the paracellular pathway transfers substances through tightly linked inter-endothelial junctions [25]. The endothelial paracellular pathway can only allow transport of molecules with a radius of less than 3 nm to move passively across the barrier [24]. The diameters of the MNPs we used in this study were measured to be typically 15–20 nm, for a generally homogeneous size [16]. When the MNPs were added to the culture media that includes serum proteins, larger agglomerates were found. Thus, these MNPs were in the size range of particles that could only pass through the endothelium by transcytosis. Our confocal microscopy image data clearly showed that the nanoparticles could be internalized in HUVECs and most of MNPs were localize within the cytoplasm (Fig. 2). Electron microscopy showed that MNPs were found outside the cell nuclei and which penetrated cells coated with vesicles, suggesting a transcytosis process (Fig. 2).

Recently, the correlation between exposure to iron oxide nanoparticles and cardiovascular diseases is of particular concern.
in nanotoxicology related fields. Nanoparticle-triggered endothelial dysfunction is hypothesized to be a dominant mechanism in the development of the diseases [26]. Endothelial cells synthesize and secrete activators and inhibitors, such as nitric oxide (NO), endothelin-1, prostacyclin, and prostaglandin to maintain the functions of vascular system and vascular barrier integrity [11,12]. Endothelial dysfunction is believed to induce pathological changes in the cardiovascular system. It can be considered as a predictor and an initiating event of atherosclerosis and its complications [26]. In our study, we found that the low concentrations of MNPs (1–200 μg/ml) did not affect the function of HUVECs. However, 400 μg/ml MNPs inhibited the cell proliferation (Fig. 1). The in vivo experiments also suggested that only the high concentration of MNPs (20 mg/kg) damaged the integrity of endothelium (Fig. 7). The loss of endothelium integrity induced by inhibition of endothelial cell proliferation might increase the vascular permeability, thus leading to an increase in the migration of monocytes and macrophages into vessels in vivo [27]. Monocyte migration and adhesion to endothelial cells are considered to be one of the early events in the initiation of atherosclerosis. Thus, the high concentration of MNPs (20 mg/kg) might have the potential danger to damage endothelium integrity and increase the vascular permeability. However, the damage of endothelium recovered after treatment with MNPs for 6 days (Fig. 7). The data indicated that MNPs at high concentration induced acute cytotoxicity in endothelial cells in the first 3 days in vivo, and then the cells would repair to normal status after 6 days by themselves.

A previous report showed that iron oxide nanoparticles at high concentration caused the dysfunction and proliferation inhibition of HUVECs by promoting NO production and depressing caveolin-1 level [28]. NO is synthesized by three mammalian NOS isoforms. Under physiological conditions, the dominant NOS isoform in the vasculature is eNOS. The activity of eNOS could be used as a sign for the functions of endothelial cells and it is an important key of vascular homeostasis [29]. In our present study, the increased NO production and elevated eNOS activity were found in HUVECs treated with 400 μg/ml MNPs, accompanied with the decreased activity of up-take Dil-Ac-LDL. It was suggested that 400 μg/ml MNPs could induce HUVEC dysfunction and inhibit cell growth in vitro. It has been known that, in the plasma membrane, eNOS is located in caveolae [30], and this location is important for eNOS activity. The localization of eNOS within the caveolae renders the enzyme inactive due to the interaction of eNOS with caveolin-1 [31]. Thus, we further investigated the level of caveolin-1.

Caveolin-1 is the principal marker of caveolae [32]. It is the inhibitor of eNOS in vivo. The activity of eNOS is inhibited by binding to caveolin-1 [33]. It is reported that the ability of caveolin-1 to inhibit eNOS may contribute to the regulation of vascular permeability. Loss of caveolin-1 markedly increases the vascular permeability through the paracellular pathway by damaging the endothelial tight junction formations [34]. In our study, the decreased level of caveolin-1 was accompanied with the increased eNOS activity both in the HUVECs in vitro and in the endothelium in vivo. At the same time, the loss of endothelium integrity.

![Fig. 7. The effect of MNPs on endothelium in aortic root of mice injected with MNPs. Arrows showed the places that were damaged.](image-url)
was accompanied with the elevation of NO production. Thus the biological reaction pathway might be as below: the MNPs were internalized in HUVECs through transcytosis process; at high concentration (400 μg/ml in vitro and 20 mg/kg in vivo), they decreased the level of caveolin-1 and increased the activity of eNOS, which induced endothelial cell dysfunction and resulted in the increased vascular permeability.

Furthermore, based on the data that the damaged endothelium and the changes of caveolin-1/eNOS/NO were only found in the mice injected with 20 mg/kg MNPs after 3 days, but it recovered to the normal level after injection for 6 days and 9 days, we deduced that the MNPs at high concentrations only induce the acute response in vivo. The body might adapt this by themselves in the long term. We will do further investigations to understand the adaptive mechanism.

5. Conclusions

In summary, the new MNPs of low concentrations (1–200 μg/ml in vitro and 5 mg/kg in vivo) have no effects on the function of HUVECs and endothelium in mice. The MNPs at high concentrations (400 μg/ml in vitro and 20 mg/kg in vivo) inhibited cell growth in vitro and damaged the endothelium in vivo, which was mediated by caveolin-1 and eNOS. This suggested that the MNPs might have potential danger when their concentration was more than 20 mg/kg in vivo.

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