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Nano-titanium dioxide induced cardiac injury in rat under oxidative stress

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ABSTRACT

Heart diseases, which are related to oxidative stress (OS), negatively affect millions of people from kids to the elderly. Titanium dioxide (TiO_2) has widespread applications in our daily life, especially nanoscale TiO_2. Compared to the high risk of particulate matter ($\leqslant$2.5 μm) in air to heart disease patients, related research of TiO_2 on diseased body is still unknown, which suggest us to explore the potential effects of nanoscale and microscale TiO_2 to heart under OS conditions. Here, we used alloxan to induce OS conditions in rat, and investigated the response of heart tissue to TiO_2 in healthy and alloxan treated rats. Compared with NMs treatment only, the synergistic interaction between OS conditions and nano-TiO_2 significantly reduced the heart-related function indexes, inducing pathological changes of myocardium with significantly increased levels of cardiac troponin I and creatine kinase-MB. In contrast with the void response of micro-TiO_2 to heart functions in alloxan treated rats, aggravation of OS conditions might play an important role in cardiac injury after alloxan and nano-TiO_2 dual exposure. Our results demonstrated that OS conditions enhanced the adverse effects of nano-TiO_2 to heart, suggesting that the use of NMs in stressed conditions (e.g., drug delivery) needs to be carefully monitored.

1. Introduction

Nowadays, mainstream nanomaterials (NMs), e.g., nano-titanium dioxide (TiO_2), are produced with the rapid development of nanotechnology. TiO_2 NMs are in close contact with people, showing a trend to replace microscale TiO_2 in foods, cosmetic, etc. (Lanza et al., 2006; Vicent and Duncan, 2006; Wickline and Lanza, 2003). Further, due to their valuable physicochemical properties, recent studies suggested that nano-TiO_2 can work as drug delivery systems and additive in pharmaceuticals (Drobne et al., 2009; Lanza et al., 2006; Shin and Lee, 2008). However, adverse effects, such as cellular dysfunction, oxidative damage, inflammatory responses, induction of thrombosis, impaired the spatial recognition memory, and liver lesions were shown *in vitro* and *in vivo* after nano-TiO_2 exposure (Cai et al., 2011; Fabian et al., 2008; Hu et al., 2010; Iavicoli et al., 2012; Li et al., 2008; Sha et al., 2011; Valant et al., 2012). In the present situation, it will be the paramount thing to consider for the safe usage of nano-TiO_2 to human beings.

As the largest cause of deaths in the world, heart diseases are no longer geriatric diseases and attack young people as well (McGill et al., 2008). The imbalance between oxidants and antioxidants can lead to oxidative stress (OS), which affects the microenvironment of cells, tissues, and organs in the body (Singal et al., 1998). Accumulating evidences show the crucial and negative roles of OS conditions in different types of heart diseases, such as heart failure, cardiovascular disease (Griendling and Alexander, 1997; Heitzer et al., 2001), hypertensive heart disease (Kadiiska

Abbreviations: ANOVA, analysis of variance; ALP, alkaline phosphatase; ALT, alanine aminotransferase; AST, aspartate aminotransferase; BW, body weight; DLS, dynamic light scattering; ELISA, enzyme linked immunosorbent assay; EDTA, ethylenedinitriletetraacetic acid; FBS, fetal calf serum; GSH, glutathione; H_2O_2, hydrogen peroxide; HRTEM, high resolution transmission electron microscopy; LDH, lactate dehydrogenase; MDA, malondialdehyde; MTT, 3-(4,5-dimethylthiazol-2-yl)-2,5-diphenyltetrazolium bromide; NMs, nanomaterials; O_2^-, superoxide anion; OD, optical density; OS, oxidative stress; PI, propidium iodide; SAED, selected area electron diffraction; SEM, scanning electron microscope; SOD, superoxide dismutase; XRD, X-ray diffraction; TiO_2, titanium dioxide.

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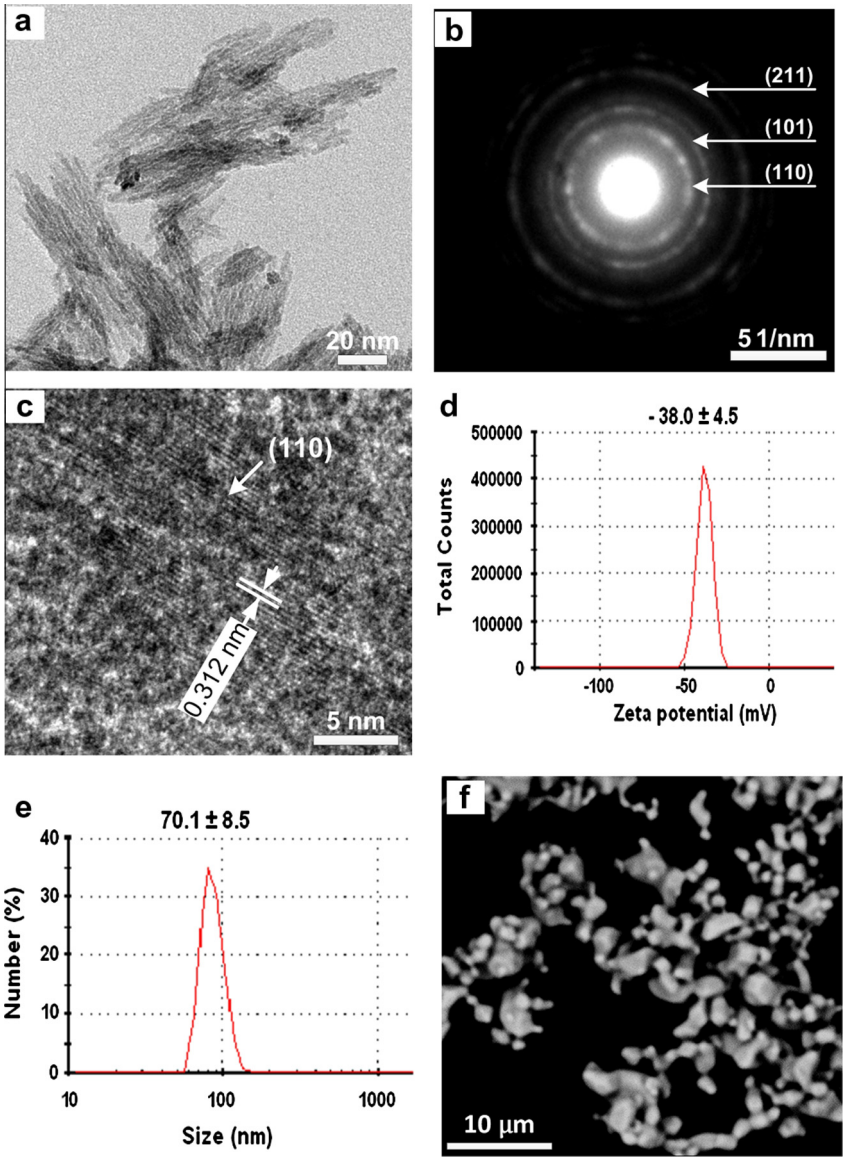

Fig. 1. Characterization of TiO_2 particles. (a) TEM micrograph of nano-TiO_2 dispersed in ethanol; (b) SAED pattern of nano-TiO_2; (c) crystal lattice plane of nano-TiO_2; (d) zeta potential of nano-TiO_2 (5 mg/mL) was the average after 12 runs of 15 measurements; (e) size distribution of nano-TiO_2 (5 mg/mL) was shown after 15 runs of 30 measurements; and (f) SEM image of micro-TiO_2.

et al., 2012), ischemic heart disease (Borillo et al., 2010), and cardiomyopathy (Cesselli et al., 2001). Compared to the high risk of particulate matter (PM, diameter $\leqslant$2.5 μm) to heart disease patients (Peters et al., 2001; Pope et al., 2006), the adverse effects of TiO_2 to heart tissue under OS conditions remain unknown, although several studies have shown that nano-TiO_2 can accumulate in healthy heart tissues *in vivo* (Chen et al., 2006; Wang et al., 2009b).

Based on the low toxicological potential and biological response of nano-TiO_2, in this study, we used alloxan to induce the artificial OS conditions, and investigated the potential synergistic effect of TiO_2 and OS conditions during cardiac injury in Sprague–Dawley (SD) rats. Compared to NMs exposed healthy rats, rats in conditions of OS showed the significantly reduction of heart rate (HR), stroke volume index (SVI), and cardiac index (CI) after nano-TiO_2 exposure, following the pathological changes of myocardium and the significantly increased levels of cardiac troponin I (cTnI) and creatine kinase-MB (CK-MB). In addition, a type of micro-TiO_2 was used as a control to study the different responses of rat hearts exposed to nanoscale and microscale TiO_2 particles, and to explore the potential synergy between nano-TiO_2 and OS conditions during cardiac injury.

2. Methods and materials

2.1. Reagent preparation

Alloxan monohydrate, nano-TiO_2 and micro-TiO_2 were purchased from Sigma–Aldrich Trading Co., Ltd., (Shanghai, China). Glutathione (GSH), brain natriuretic peptide (BNP), cardiac troponin I (cTnI), creatine kinase-MB (CK-MB), and myoglobin (MYO) enzyme linked immunosorbent assay (ELISA) kits were obtained from Roche Ltd., (Shanghai, China), R&D Systems and Jiancheng Bioengineering Company (Nanjing, China).

2.2. Characterization of TiO_2

To avoid contamination during rat experiments, TiO_2 was firstly sterilized through 140 °C dry heat for 5 h. Dry heat sterilized TiO_2 was suspended in 0.9% saline solution and 30 min ultrasonic treatment in advance before rat experiments. Morphology, selected area electron diffraction (SAED) pattern and crystal lattice plane of sterilized nano-TiO_2 were checked using high resolution transmis-

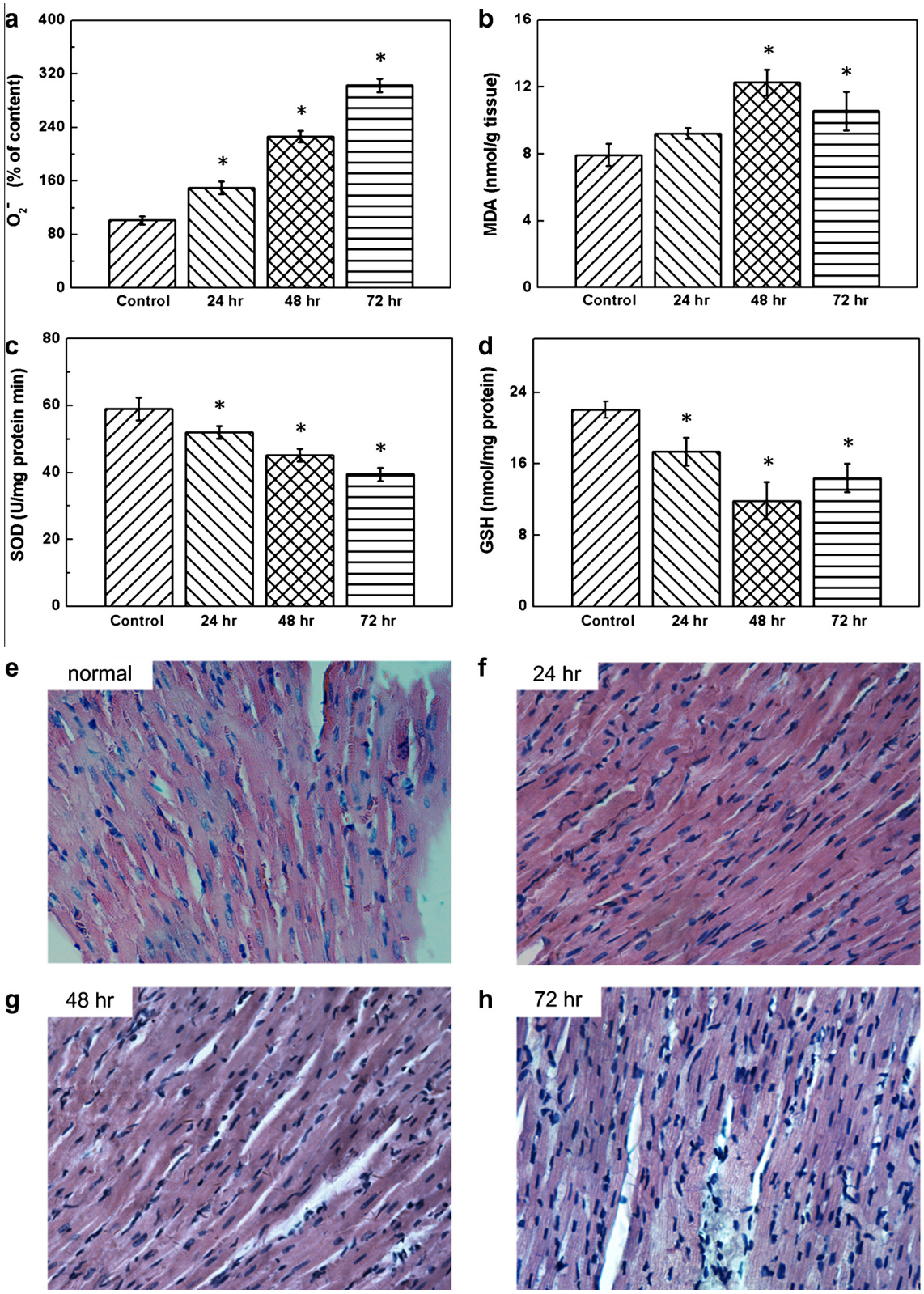

Fig. 2. Detection of OS conditions in rat heart after alloxan treatment. (a–d) Changes of O_2^-, MDA, SOD and GSH levels in rat heart after 24–72 h alloxan injection. Values were mean ± SD, $n = 6$. $^*P < 0.05$, compared with the relative control (one-way ANOVA, Tukey's *post hoc* test). (e) HE stained sections (400×) of rat left ventricular inner myocardium in group 1, without significant pathological changes. (f–h) HE stained sections (400×) of myocardium in group 2 after 24, 48 and 72 h alloxan injection, without significant pathological changes.

sion electron microscopy (HRTEM, JEOL JEM-2100F). Size distribution and zeta potential of nano-TiO_2 were performed through Zetasizer Nano ZS90. Brunauer–Emmett–Teller method was used to measure the surface area of nano-TiO_2. The morphology of micro-TiO_2 was checked using scanning electron microscope (SEM).

2.3. Rat groups

All animal experiments and procedures used in this study were approved by the Ethics Committee of Animal Experiments of Xi'an Jiaotong University (Permit Number: XA-20121010), according to the recommendations in the Guide for the Care

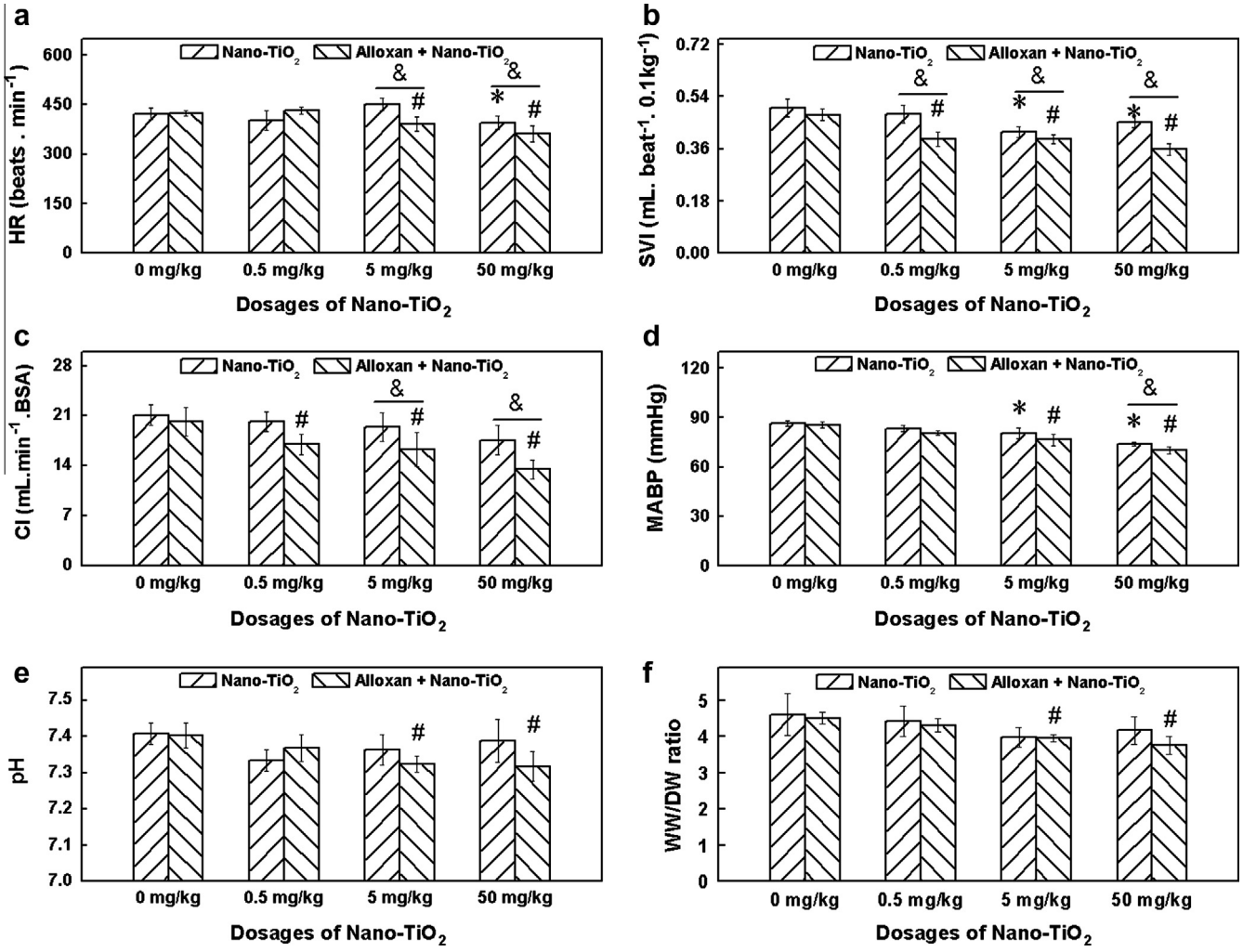

Fig. 3. Effects of nano-TiO_2 particles on physiological variables of heart in healthy and alloxan treated rats. (a) HR (heart rate), (b) SVI (stroke volume index), (c) CI (cardiac index), (d) MABP (mean arterial blood pressure), (e) pH, and (f) WW (wet weight)/DW (dry weight) ratio. Data were shown as mean ± SD, $n = 6$. $^{*}P < 0.05$, comparing with group 1 (one-way ANOVA, Tukey's *post hoc* test); $^{\#}P < 0.05$, comparing with group 2 (one-way ANOVA, Tukey's *post hoc* test); $^{\&}P < 0.05$, group 3 versus group 6, group 4 versus group 7, group 5 versus group 8 (Student's *t*-test).

Table 1
Two-way ANOVA analysis on physiological variables of heart in healthy and alloxan treated rats after nano-TiO_2 exposure.

Treatment	HR	SVI	CI	MABP	pH	WW/DW ratio
Alloxan	$F_{(1,40)} = 7.56$ $P = 0.009$	$F_{(1,40)} = 1.70$ $P = 0.200$	$F_{(1,40)} = 0.51$ $P = 0.681$	$F_{(1,40)} = 12.99$ $P = 0.001$	$F_{(1,40)} = 8.25$ $P = 0.006$	$F_{(1,40)} = 2.98$ $P = 0.092$
Nano-TiO_2	$F_{(3.40)} = 14.11$ $P < 0.001$	$F_{(3.40)} = 6.94$ $P = 0.001$	$F_{(3.40)} = 10.65$ $P < 0.001$	$F_{(3.40)} = 72.03$ $P < 0.001$	$F_{(3.40)} = 5.24$ $P = 0.004$	$F_{(3.40)} = 9.73$ $P < 0.001$
Alloxan × nano-TiO_2	$F_{(3.40)} = 11.60$ $P < 0.001$	$F_{(3.40)} = 18.93$ $P < 0.001$	$F_{(3.40)} = 10.55$ $P = 0.002$	$F_{(3.40)} = 0.89$ $P = 0.455$	$F_{(3.40)} = 0.22$ $P = 0.882$	$F_{(3.40)} = 0.86$ $P = 0.472$

Note: $P < 0.05$ was considered statistically significant.

and Use of Laboratory Animals in our institutes. Sprague–Dawley (SD) rats, acquired from The Fourth Military Medical University (Xi'an, China) with body weight (BW) of 180–215 g, were fed using sterilized water and food at room temperature (25 °C) and 60% relative humidity. Healthy male rats were divided into 14 groups with 6 rats in each group, which were slowly and gently administered with alloxan monohydrate and TiO_2 via intramuscular injection and intraperitoneal route, respectively. These groups included group 1 (for normal), group 2 (70 mg/kg BW of alloxan) (Sciences et al., 2007), group 3 (0.5 mg/kg BW of nano-TiO_2), group 4 (5 mg/kg BW of nano-TiO_2), group 5 (50 mg/kg BW of nano-TiO_2), group 6 (0.5 mg/kg BW of nano-TiO_2 for 48 h after 24 h injection of 70 mg/kg BW alloxan), group 7 (5 mg/kg BW of nano-TiO_2 for 48 h after 24 h alloxan injection), group 8 (50 mg/kg BW of nano-TiO_2 for 48 h after 24 h alloxan injection), groups 9–11 (0.5, 5, and 50 mg/kg BW of micro-TiO_2), and groups 12–14 (0.5, 5, and 50 mg/kg BW of micro-TiO_2 for 48 h after 24 h alloxan injection). In addition, diabetes mellitus was induced by 70 mg/kg BW alloxan after 72 h injection in rats (Sciences et al., 2007). To avoid the potential effect of alloxan to heart tissues, the longest period of alloxan injection in rats was 72 h. Based on the period that alloxan led to OS conditions (24 h), the acute exposed period of TiO_2 was set at 48 h in related groups.

At the end of all experiments, intraperitoneal injection of pentobarbital (45 mg/kg BW) was used to anesthetize rats. Rat whole blood, which obtained via femoral artery catheterization, was centrifuged at 2000*g* for 20 min to collect sera. Heart tissue was excised for weight, measurement of OS indicators, and histopathological assay.

2.4. OS indicators

The levels of OS endpoints (superoxide anion (O_2^-), malondialdehyde (MDA), superoxide dismutase (SOD) and GSH) in heart were measured immediately after homogenization and centrifugation of the excised tissues. The generation of O_2^-

was monitored through its ability to convert dihydroethidium to ethidium bromide. The myocardial lipid peroxidation was assessed through MDA content using thiobarbituric acid reactive substances (TBARSs) (Buege and Aust, 1978). As an antioxidant enzyme, the activity of SOD was tested through inhibiting the photochemical reduction of nitroblue tetrazolium to blue formazan (Kuo et al., 2011). GSH content was estimated using the commercial ELISA kit, which can react with 5,50-dithiobis (2-nitrobenzoic acid) and generate 2-nitro-5-thiobenzoic acid and glutathione disulfide (GSSG) (Wang et al., 2009a).

2.5. Cardiac physiological parameters

Heart rate (HR), cardiac output (CO), and mean arterial blood pressure (MABP) were monitored using Biopac MP150 system (Biopac System Inc., USA). Blood pH was assessed through Compact 3 blood gas analyzer (Roche Diagnostics Ltd.). Stroke volume index (SVI) = (CO/HR)/BSA, where BSA means body surface area (Rodeheffer et al., 1984). Cardiac index (CI) was calculated as CO/BW (Mulder et al., 2002). The wet weight (WW) of heart was weighed immediately using electronic balance after heart removal, whilst the corresponding dry weight was measured after the heart was completely dried (100 °C/24 h). WW/DW meant the ratio of WW to DW.

2.6. Cardiac injury detection

For histopathological assay, removed hearts were fixed by 10% phosphate buffered formalin, processing routinely into paraffin, section-cut, and hematoxylin and eosin (HE) staining. Digital photos of heart tissue (left ventricular inner myocardium) sections were obtained using Axiocam HRc microscopy systems (Carl Zeiss Inc., Germany). As the biomarkers of cardiac injury, the levels of BNP, cTnI, CK-MB and MYO in sera were measured in accordance with previously literatures (Crisman et al., 1987; Heidrich et al., 2008; Wang et al., 2011; Yavuz et al., 2008).

2.7. Statistical analysis

Data were shown as mean ± standard deviation (SD). Two-tailed Student's *t*-test, one-way analysis of variance (ANOVA) with Tukey's *post hoc* test, and two-way ANOVA were used wherever appropriate. *P*-values less than 0.05 indicated the statistical significance.

3. Results

3.1. Characterization of TiO_2

To characterize the nano-TiO_2 used in this study, we checked the morphology using TEM, Fig. 1a. We observed that the nano-TiO_2 was rod-like with an average size of 15 nm × 60 nm (diameter × length). The SAED pattern suggested that nano-TiO_2 was in the rutile phase (Fig. 1b). The d-spacing of crystal lattice plane (1 1 0) was determined as 0.312 nm (Fig. 1c). Further, the nano-TiO_2 carried negative charges (Fig. 1d) with surface area of 150–171 m^2/g. Fig. 1e was the size distribution of nano-TiO_2 in 0.9% normal saline suspension. The shape of rutile micro-TiO_2 was showed in Fig. 1f with sizes of 1–5 μm.

3.2. Alloxan induced OS conditions in rat heart

To investigate the OS conditions as induced by alloxan treatment (70 mg/kg BW, 24–72 h), we assessed the changes of OS endpoints in heart tissues. Significant increase in the O_2^- (Fig. 2a) and MDA (Fig. 2b) levels and reduction in contents of SOD (Fig. 2c) and GSH (Fig. 2d) were shown after 24 h alloxan injection. Further, compared to normal HE images of heart sections (Fig. 2e), we did not observe significant pathological changes induced by alloxan, even after 72 h treatment (Fig. 2f–h). All these results indicated that alloxan (70 mg/kg BW) could lead to OS conditions in rats after 24 h injection without causing obvious cardiac injury. We then chose this condition to study the adverse effect of TiO_2 in rats under OS conditions.

3.3. Physiological variables of rat heart

To explore the potential effects on heart-related function after 48 h TiO_2 exposure, we monitored the changes in physiological variables of heart, including HR, SVI, CI, MABP, pH and WW/DW ratio (Fig. 3). Compared to controls, we observed significant decrease in HR when the doses of nano-TiO_2 were above 5 and 50 mg/kg BW for alloxan treated and healthy rats, respectively (Fig. 3a). Further, compared with NM exposure only, OS conditions resulted in lower HR in alloxan treated groups after exposed 5 mg/kg BW NMs. Mild (90–97% of peak value) to severe (67–75% of peak value) reductions of SVI (Fig. 3b), CI (Fig. 3c) and MABP (Fig. 3d) were also obtained after nano-TiO_2 exposure in both healthy and alloxan treated rats. Significant differences of SVI, CI, and MABP between NM and alloxan-NM treated groups were shown when the doses of NMs were higher than 5, 5, and 50 mg/kg BW, respectively. Enhanced reduction in pH values (Fig. 3e) and slightly decreased WW/DW ratio (up to 9% of control, Fig. 3f) were revealed in groups 7–8. Further, two-way ANOVA exhibited significant effects of nano-TiO_2 to all above six variables, and significant interactions between alloxan and nano-TiO_2 to the changes of HR, SVI, and CI (Table 1). These results suggested that OS conditions enhanced the adverse effects of nano-TiO_2 to cardiac physiological variables, showing a synergic effect between OS conditions and nano-TiO_2. However, micro-TiO_2 showed no significant effect to the six physiological variables of heart in both healthy and alloxan treated rats in groups 9–14 (data not shown).

3.4. Histopathological assay and assessment of cardiac injury biomarkers

To explore the potential explanation for diminished cardiac physiological variables after nano-TiO_2 exposure, we checked the pathological sections of heart tissues in groups 3–8 (Fig. 4). For the healthy rats, myocardium showed normal appearance after 48 h nano-TiO_2 exposure, even if the maximum dose of NMs (50 mg/kg BW) was applied (Fig. 4b and c). Compared to groups 4–5, we observed the slight (Fig. 4e, swollen myocytes, group 7) to severe (Fig. 4f, vacuolar degeneration and myocardium necrosis, group 8) pathological changes in alloxan treated rats after exposed to 5 and 50 mg/kg BW nano-TiO_2. Further, we measured the levels of BNP, cTnI, CK-MB, and MYO in rat sera to assess cardiac injury. In contrast with healthy rats, we did not observe significant changes of all above cardiac injury biomarkers in healthy rats with the increasing doses of nano-TiO_2 (Fig. 4g–j). However, nano-TiO_2 (5 and 50 mg/kg BW) induced significantly enhanced levels of cTnI and CK-MB in alloxan treated rats (Fig. 4h and i), and obvious difference of these two biomarkers between healthy and alloxan treated rats. For the changes of cTnI level, two-way ANOVA revealed a significant effect of nano-TiO_2 ($F_{(3,40)} = 10.319$, $P = 0.003$) but not alloxan ($F_{(1,40)} = 2.294$, $P = 0.093$), and a significant interaction ($F_{(3,40)} = 60.931$, $P < 0.001$). In addition, the significant interaction of alloxan and nano-TiO_2 ($F_{(3,40)} = 14.187$, $P < 0.001$) on CK-MB also showed after two-way ANOVA analysis. Thus, OS conditions synergistically led to pathological changes of myocardium and the enhanced levels of cardiac injury biomarkers after above 5 mg/kg BW of nano-TiO_2 exposure, which was in accordance with changes of cardiac physiological variables (Fig. 4).

3.5. Changes of OS endpoints in rats after TiO_2 exposure

To explore the potential mechanism of cardiac injury after nano-TiO_2 exposure, we measured the OS endpoints in groups 3–14. Micro-TiO_2 did not lead to the significant changes of O_2^-, MDA, SOD, and GSH values in both healthy (Fig. S1) and alloxan treated (Fig. 5) rats, even after 50 mg/kg BW exposure. For the

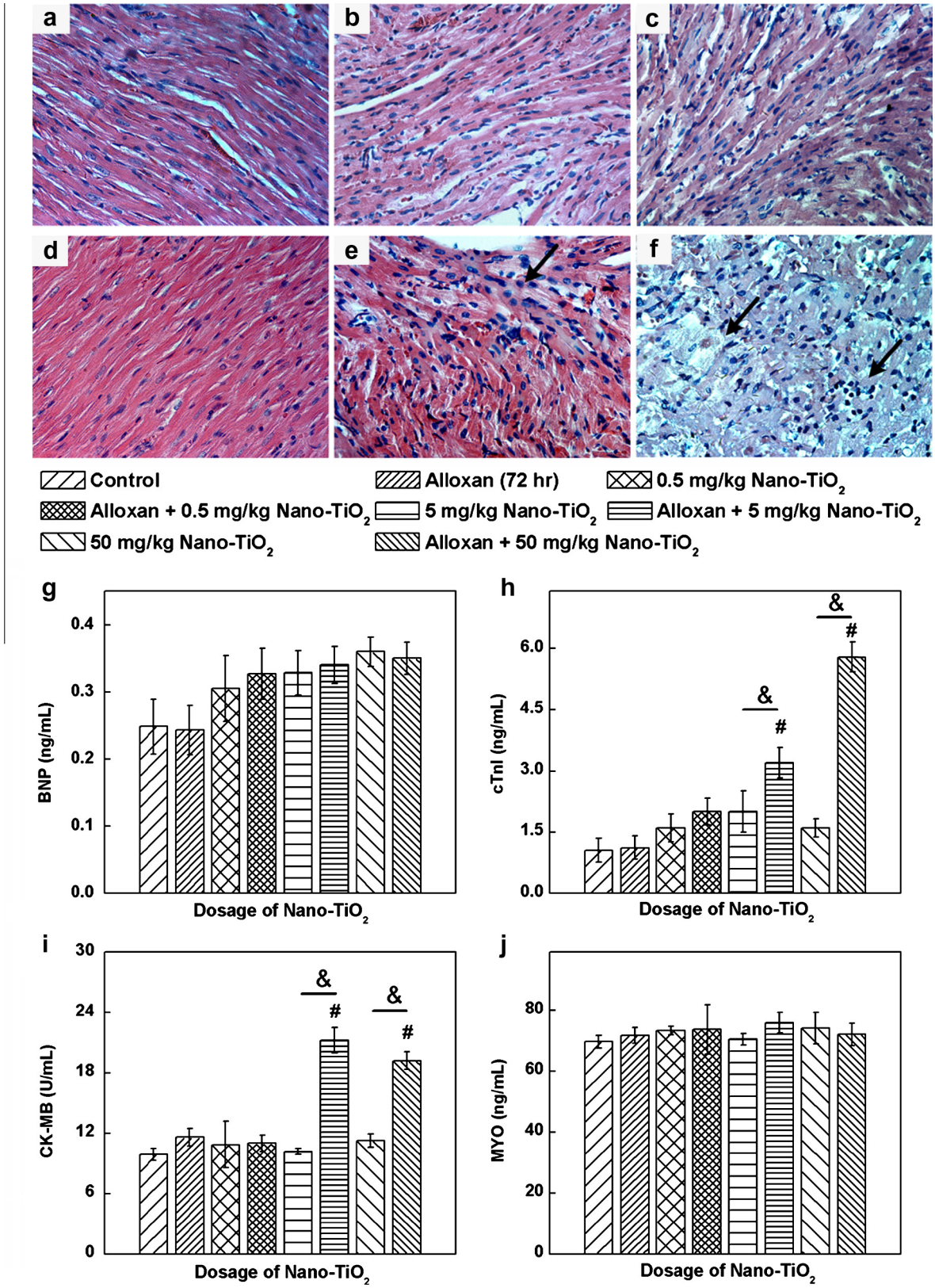

Fig. 4. Effects of nano-TiO_2 particles upon HE stained sections of rat left ventricular inner myocardium and changes of cardiac injury biomarkers in healthy and alloxan treated rats. (a) Healthy rats after 0.9% saline injection: normal appearance of myocardium (400×). (b) Healthy rats after 5 mg/kg nano-TiO_2 exposure: normal appearance of myocardium (400×). (c) Healthy rats after 50 mg/kg nano-TiO_2 exposure: normal appearance of myocardium (400×). (d) Alloxan treated rats without nano-TiO_2 exposure: normal appearance of myocardium (400×). (e) Alloxan treated rats after 5 mg/kg nano-TiO_2 exposure: acute myodegeneration with swollen myocytes (arrow, 400×). (f) Alloxan treated rats after 50 mg/kg nano-TiO_2 exposure: vacuolar degeneration and myocardium necrosis (arrow, 400×). As biomarkers of cardiac injury, (g) BNP, (h) cTnI, (i) CK-MB, and (g) MYO levels were measured. Values represent mean ± SD, $n = 6$. $^{\#}P < 0.05$, comparing with the group 2 (alloxan, 72 h) (one-way ANOVA, Tukey's *post hoc* test); $^{\&}P < 0.05$, group 4 versus group 7, group 5 versus group 8 (Student's *t*-test).

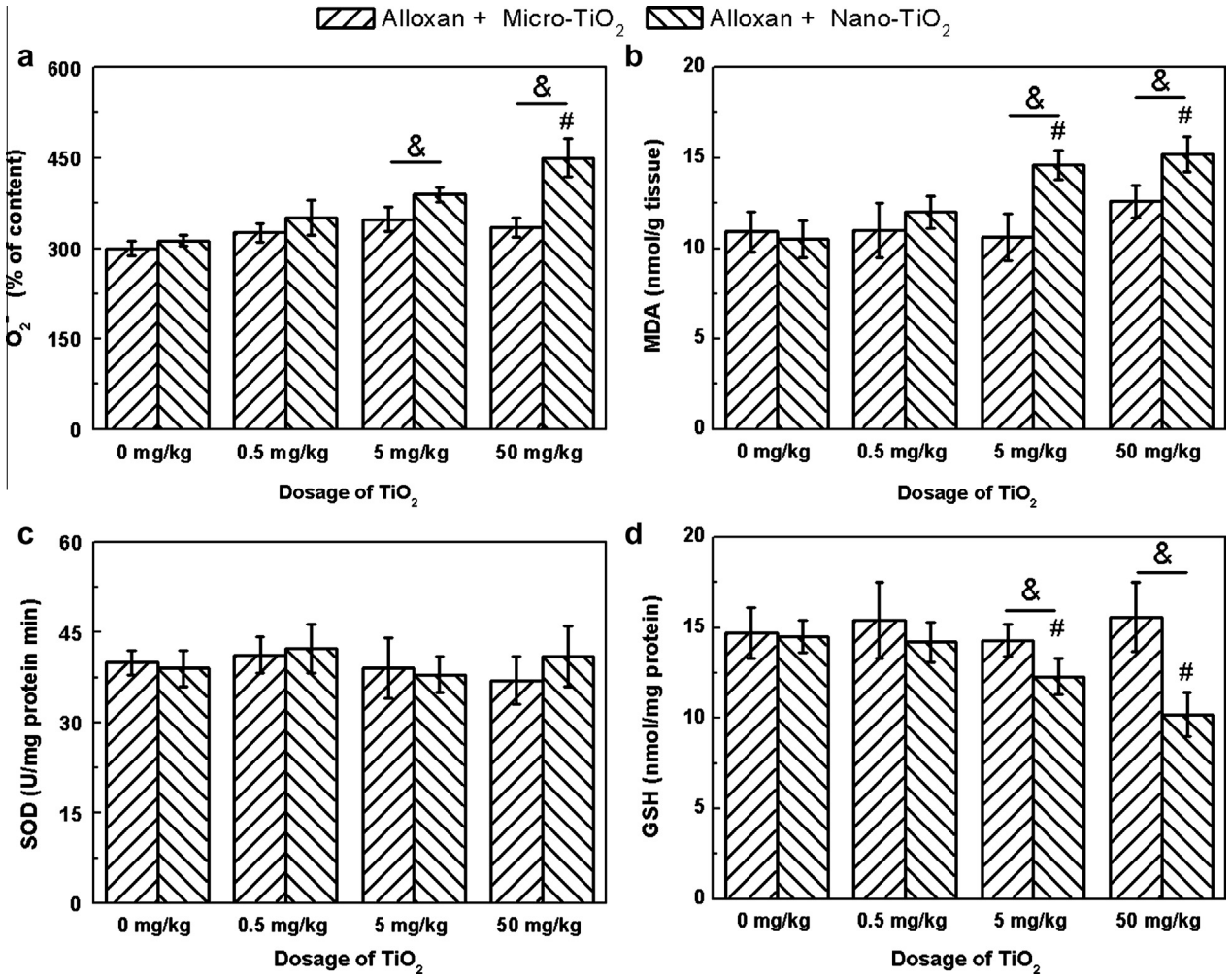

Fig. 5. Changes of OS endpoints in alloxan treated rats after TiO_2 exposure. (a) O_2^-, (b) MDA, (c) SOD, and (d) GSH. Values were mean ± SD, $n = 6$. $^{\#}P < 0.05$, comparing with the group 2 (alloxan, 72 h) (one-way ANOVA, Tukey's *post hoc* test); $^{\&}P < 0.05$, group 7 versus group 13, and group 8 versus group 14 (Student's *t*-test).

healthy rats, 48 h exposure of nano-TiO_2 showed no effect to above OS endpoints (Fig. S1). However, a significant increase in the levels of O_2^- (Fig. 5a) and MDA (Fig. 5b) and a significant decrease in GSH content (Fig. 5d) were observed in nano-TiO_2 exposed rats after 72 h alloxan injection. Moreover, data of O_2^-, MDA, and GSH after nano-TiO_2 and micro-TiO_2 exposure were showed significant difference when the doses were higher than 5 mg/kg BW. These results suggested that nano-TiO_2 aggravated the OS conditions in alloxan treated rat hearts, which might play an important role during cardiac injury.

4. Discussions

In this study, we assessed the adverse effects of TiO_2 to heart tissues in healthy and alloxan treated rats. Micro-TiO_2 showed no adverse effect to the heart functions in both healthy and alloxan treated rats. Compared to nano-TiO_2 exposed healthy rats, significant decrease in heart-related functions (HR, SVI, and CI) (Fig. 3), obvious pathological changes in heart tissues, and increased levels of cTnI and CK-MB (Fig. 4) were shown in rats under OS conditions. Nano-TiO_2 aggravated the OS conditions in alloxan treated rats (Fig. 5), suggesting nano-TiO_2 and OS conditions synergistically provoked serious injury to heart tissues.

The minimum dose of NMs exhibited the risk to heart of alloxan treated rats was 5 mg/kg BW, which was less than the dose used in healthy mice (2592 mg/kg BW) (Chen et al., 2009) and rats (560–1000 mg/kg BW) for acute adverse effect researches (Fabian et al., 2008; Warheit et al., 2007). Further, the minimum dose was still lower than the permissible exposure limit (PEL) of occupational safety and health administration (15 mg/m^3) (Zhang et al., 2011). For a 60 kg sick human, this dose only equals to 0.3 g and 20 m^3 by minimum dose and PEL, respectively. Therefore, the adverse effects of nano-TiO_2 on patients should be paid more attention.

For the changes of HR after nano-TiO_2 exposure only, TiO_2 NMs can obviously reduce contraction amplitude of normal adult rat ventricular cardiomyocytes and human embryonic stem cell-derived cardiomyocytes *in vitro* (10–100 μg/mL) (Jawad et al., 2011), leading to the decrease of HR in healthy *Daphnia magna* *in vivo* (2 ppm) (Lovern et al., 2007). Our result showed not only the reduction of HR in healthy rats after 50 mg/kg BW of nano-TiO_2 exposure but also the enhanced adverse effect in present of OS conditions, because OS conditions can affect the regularity of heartbeat (Custodis et al., 2008) and exhibit a synergic effect with nano-TiO_2 in this study (Table 1).

Compared with groups 4–5, SVI and CI were significantly reduced in alloxan treated rats (Fig. 3b and c). CO is the common factor amongst SVI and CI changes (Mulder et al., 2002; Rodeheffer et al., 1984), and the significant reduction in CO was obtained in OS rats after 5 and 50 mg/kg BW nano-TiO_2 exposure in comparison with groups 4–5 (Fig. S2). The synergistic effects between OS

conditions and NMs might be weakening the cardiac pumping force for the following reasons. First, the diminished HR can lead to low CO if without a compensating stroke volume (Rodeheffer et al., 1984). Second, significant myocardium necrosis often leads to reduced CO due to its influence on cardiac contractile function (Doty et al., 1974; Horton et al., 1995), which was consistent with severe pathological changes in groups 7–8, such as swell and necrosis of myocardium (Fig. 4e and f). In comparison with groups 4–5, nano-TiO_2 significantly increased the levels of cardiac injury biomarkers in groups 7–8 (Fig. 4h and i). cTnI is a sensitive inductor to specific assessment of congestive heart failure (Missov et al., 1997). Abnormally high CK-MB level is intensively related to acute myocardial infarction (Kavsak et al., 2007). Third, significant decrease in blood pH value (Fig. 3e), which obtained in groups 7–8, can kill cardiac myocytes (Bond et al., 1991). Fourth, the enhanced generation of ROS is one of the most widely accepted toxic mechanisms of nano-TiO_2 (Long et al., 2007), which can affect biological antioxidant responses and can induce OS condition (Finkel and Holbrook, 2000). Compared to void response of micro-TiO_2 in alloxan treated groups, nano-TiO_2 induced significant changes of O_2^-, MDA, and GSH, suggesting that nano-TiO_2 aggravated the OS conditions of heart (Fig. 5). The accumulated OS conditions can result in weak cardiac output, cardiac injury, and further heart diseases (Borillo et al., 2010; Cesselli et al., 2001; Kadiiska et al., 2012).

In our experiments, TiO_2 NMs were injected into rats via intraperitoneal administration, which can cross small intestine and distribute into heart (Hillyer and Albrecht, 2001). Based on our focus, some people might suggest that intravenous injection of nano-TiO_2 is a good choice. The exposure route of NMs might be a major limit in this study, which will be remedy in the following relative researches. Here, we thought that intraperitoneal administration is available for the nanotoxicity assessment to heart, because clinical trials demonstrated that drug delivery via intraperitoneal route is well tolerated, efficient, and feasible. However, the components in serum can increase the size of nano-TiO_2, and aggregation of NMs may pump into vein to provoke a deadly embolism (Galeone et al., 2012; Maiorano et al., 2010; Yamaguchi et al., 2010).

5. Conclusion

In conclusion, we compared adverse effects of nanoscale and microscale TiO_2 in healthy and alloxan treated rats. In contrast to the non-toxic effect of micro-TiO_2, we found that NMs in conjunction with OS conditions synergistically led to significant decrease in HR, SVI, and CI, as well as increased levels of cTnI and CK-MB in distinct pathological heart tissues compared to NMs only exposed rats. The cardiac injury might be induced by the aggravation of OS conditions after alloxan and nano-TiO_2 dual treatment. This study emphasized that adverse effects of NMs should be evaluated under OS conditions rather than under healthy conditions only.

Conflict of Interest

The authors declare that there are no conflicts of interest.

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Appendix A. Supplementary material

Supplementary data associated with this article can be found, in the online version, at http://dx.doi.org/10.1016/j.fct.2013.04.050.

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