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IN VITRO AND *IN VIVO* BIOCOMPATIBILITY STUDIES OF ZNO NANOPARTICLES

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The *in vitro* and *in vivo* biocompatibility of the ZnO nanoparticles were evaluated using cell model and animal model, by MTT assay, flow cell cytometry, pathological optical and electron microscopy examinations. Both the L929 cell and Hela cell proliferative activity were strongly inhibited by the presence of ZnO nanoparticles, no matter cultured with low dose and high dose suspension. AnnexinV-FITC/PI-FCM assay showed that the number of necrotic cell and apoptotic cell increased significantly. Feeding the ZnO nanoparticle suspension through digestive tract would lead to the damage to some primary organs (heart, lung, liver and kidney). Further investigation by TEM showed the expanded sarcoplasmic reticulum and organelle vacuolation features.

Keywords: ZnO; biocompatibility; cell proliferation; apoptosis.

1. Introduction

Being a key functional material with versatile properties, such as dual semiconducting and piezoelectric properties, ZnO has important applications in optoelectronic devices, sensors, lasers, transducers, and photovoltaic devices[1-2]. ZnO nanoparticles have been used in many applications in our daily life, such as drug carriers and cosmetics[3]. However, although inhalation of ultrafine ZnO particles at relatively high dose (500 mg/m3) for 2 hours did not induce acute systemic effects in humans, inhalation of ZnO fumes in an occupational setting can cause metal fume fever (fatigue, chills, fever, myalgias, cough, dyspnea, leukocytosis, metallic taste, and salivation)[4]. The interaction of ZnO nanomaterial with the organism is still far known from the viewpoint of biomaterial scientist. The objective of the present study is to evaluate both the *in vitro* and *in vivo* biocompatibility of the ZnO nanoparticles using cell model (both mouse fibroblast and human cervical cancer cells) and animal model (ICR mouse).

2. Experimental Procedure

ZnO, with mean diameter 20nm, as identified by TEM and HREM in Fig. 1, were made by Shanghai Huijing Sub-Nano scale New Material Company, LTD. The ZnO

nanoparticles was distributed into RPMI 1640 culture medium containing 10% fetal bovine serum (FBS) to become 400 μg/ml suspension, then were high temperature & pressure sterilized and ultrasonically vibrated for 10 minutes before use.

L929 and Hela cells were cultured in RPMI1640 supplemented with 10% fetal bovine serum and 1% L-glutamine, penicillin, and streptomycin. The MTT (3-(4,5-dimethyl-2-thiazolyl)-2,5-diphenyl-2H-tetrazolium bromide) assay was used to evaluate proliferative activity. Exposure to 50 μl RPMI1640 was used as a negative control and 50 μl DMSO as a positive control. At least three time points are depicted for each assay.

30 ICR mice, male, aged 6-8 weeks and weighing 18-25g, were used in the experiment, with 15 each for control group and ZnO (20nm) group. The 30mg/ml suspension was administered at a dose of 0.2ml through digestive tract, each time a day and lasted 7 days. The animals were observed if they were illed or died during the experiment, and at day 17 were killed by exsanguinations following pelltobarbitalum natricum anaesthesia. The blood was conserved by adding heparin. All animals were examined grossly. Tissues including liver, kidney, heart, spleen, lung and brain were collected. Moiety were fixed in a 10% formaldehyde solution and others were freezed in -20°C. After fixation, tissues were routinely processed, embedded in paraffin, cut at a microtome setting of 5 μm, mounted on glass slides, stained with hematoxylin and eosin and examined by light microscopy. After dehydration and resin embedding, some tissues were sectioned to 70nm thick, stained with uranyl acetate and lead citrate and examined by JEM-100 CX II transmission electron microscopy. The animal experiment was performed in accordance with the Guide for the Care and Use of Laboratory Animals, with the approval of the Beijing Municipal Science and Technology Committee.

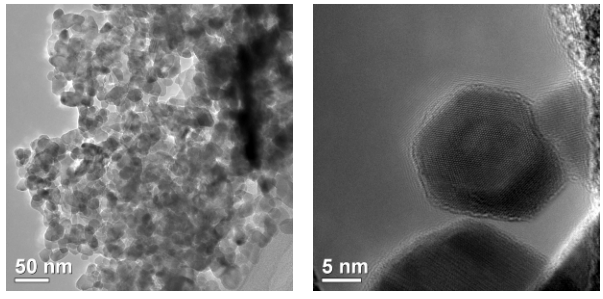

Fig. 1. TEM bright image and HREM image of the ZnO nanoparticles.

3. Results and Discussion

3.1. *In vitro biocompatibility evaluation of ZnO nanoparticles*

Table 1 and Table 2 listed the optical density (OD) and relative growth rate (RGR) of L929 cells and Hela cells based on the results of MTT assay. It can be seen that both the L929 cell and Hela cell proliferative activity were strongly inhibited by the presence of nano ZnO, no matter at low dose and high dose. The RGR values were quite different

from the negative control (normal cell) and was evidently similar to the positive control (DMSO)($p<0.05$), which indicates the heavy cytotoxicity of ZnO nanoparticles.

Table 1. The optical density (OD) and relative growth rate (RGR) of L929 cells based on the results of MTT assay.

Group/Concentration		24h		48h	
		High	Low	High	Low
Normal L929 cell	OD	0.3909		0.9446	
	RGR	100%		100%	
ZnO (20nm)	OD	0.0345	0.0237	0.0430	0.0400
	RGR	8.83%	6.07%	4.55%	4.23%
12%DMSO	OD	0.030		0.017	
	RGR	7.674%		1.78%	

Table 2. The optical density (OD) and relative growth rate (RGR) of Hela cells based on the results of MTT assay.

Group/Concentration		24h		48h	
		High	Low	High	Low
Normal Hela cell	OD	0.5839		0.774	
	RGR	100%		100%	
ZnO (20nm)	OD	0.0871	0.1022	0.107	0.118
	RGR	14.91%	17.51%	13.82%	15.24%
12%DMSO	OD	0.046		0.090	
	RGR	7.88%		11.63%	

Figures 2 and 3 illustrated the early L929 cell and Hela cell apoptosis percentages detected by AnnexinV-FITC/PI –FCM assay at 24h and 48h. The dot lied in the lower left quadrant, lower right quadrant, upper right quadrant and upper left quadrant represents the living cell, apoptotic cell, secondary necrotic cell and mechanical destruction of cell during culture procedure, respectively. For ZnO nanoparticle group, the percentage of necrotic cell is the dominated for both the L929 cell and Hela cell, with over 60% at 24h and over 80% at 48h. The following is the percentage of the apoptotic cell. Figure 4 took the Hela cell after cultured in ZnO (20nm) suspension for 24h as example to show the typical morphologies under TEM, and both condensed new moon-type chromatin and irregular organelle morphology are observed.

3.2. *In vivo biocompatibility evaluation of ZnO nanoparticles*

Figure 5 showed the light optical image of the mouse organs including heart, lung, liver, spleen, kidney and brain. The spleen and brain cells are normal in comparison to the blank group, whereas the heart, lung, liver and kidney cells turned abnormal. Cytoplasmic coagulation and inflammatory cell infiltration happened in the heart, few lymphocyte infiltration and broadened alveolar septum could be found in the lung, hydropic degeneration could be observed in the liver, and glomerular swelling and tubular swelling was obvious in the kidney.

Figure 6 illustrated the TEM images of the cardiac tissue(a)-(b), liver tissue(c)-(d) and brain tissue(e)-(f) of the experimental mouse. Expanded sarcoplasmic reticulum and vacuolar degeneration could be found in the cardiac tissue, as shown in Fig. 6(a) and Fig. 6(b). Organelle vacuolation is the typical feature of the liver tissue, as indicated in Fig. 6(c) and Fig. 6(d). Loose axon, organelle vacuolation and vacuolar degeneration could be observed inside the brain tissue, as illustrated in Fig. 6(e) and Fig. 6(f).

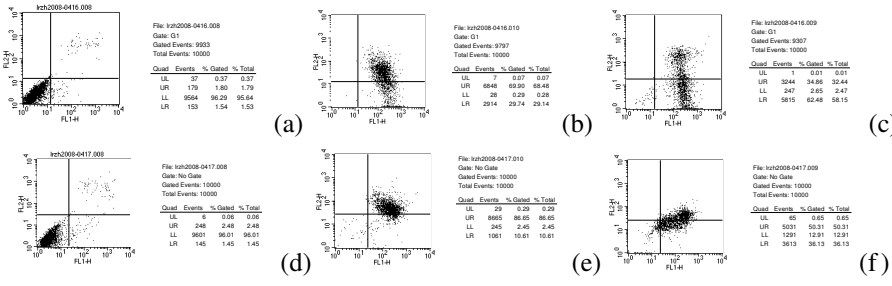

Fig. 2. AnnexinV-FITC/PI -FCM assay on L929 cell. (a) blank control, 24h; (b) ZnO (20nm), 24h; (c) DMSO, 24h; (d) blank control, 48h; (e) ZnO (20nm), 48h; (f) DMSO, 48h.

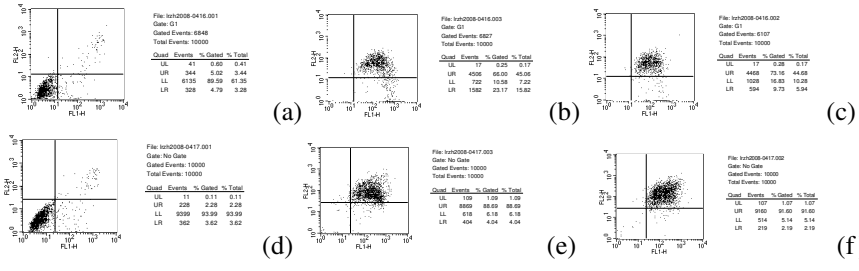

Fig. 3. AnnexinV-FITC/PI -FCM assay on Hela cell. (a) blank control, 24h; (b) ZnO (20nm), 24h; (c) DMSO, 24h; (d) blank control, 48h; (e) ZnO (20nm), 48h; (f) DMSO, 48h.

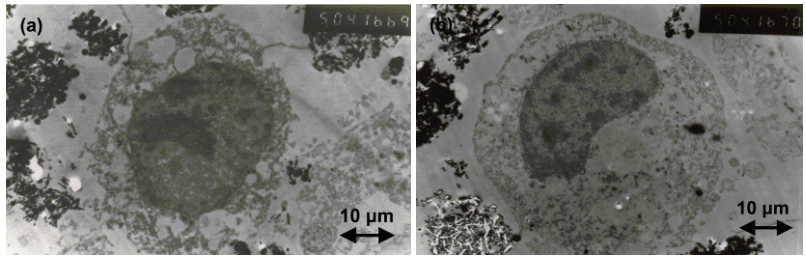

Fig. 4. TEM image of Hela cell after cultured in ZnO (20nm) suspension for 24h showing the typical morphologies (a) condensed new moon-type chromatin and (b) irregular organelle morphology.

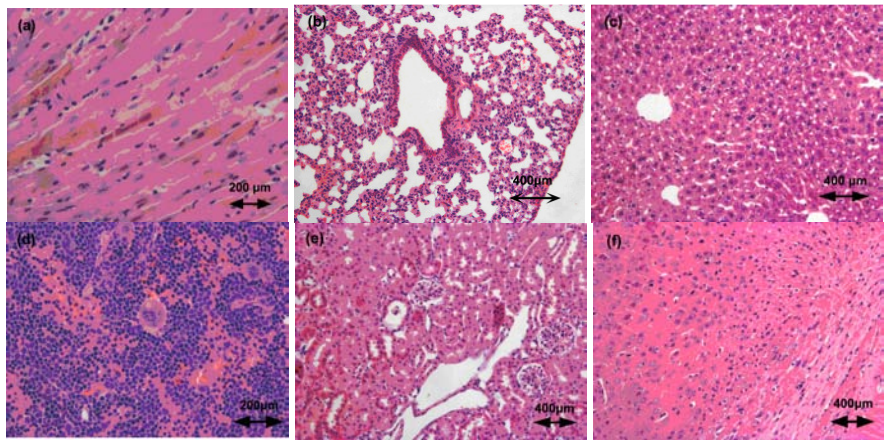

Fig. 5. Light microscopic observation of the organ of the mouse after feeding ZnO(20nm) nanoparticle suspension (a) heart; (b), lung; (c) liver; (d) spleen; (e) kidney; (f) brain.

Accurately assessing the toxicity and safety of ZnO nanomaterials is of utmost importance. On the one hand, the present work shows that the ZnO nanoparticles with the diameter exhibit toxicity both *in vitro* and *in vivo*, and it suggests that the further application of ZnO nanoparticles in the body fluid should be cautious. Dechsakulthorn et al.[5] assessed the cytotoxicity of ZnO nanopowder with particle size 50-70nm in human skin fibroblasts using the colourimetric MTS (3-(4,5-dimethylthiazol-2-yl)-5-(3-carboxymethoxyphenyl)-2-(4-sulfophenyl)-2Htetrazolium) *in vitro* assay, and his work indicated higher toxicity of ZnO at both 4 and 24 h. Lin et al.[6] had also demonstrated that 70nm and 420nm ZnO particles significantly reduce cell viability and cause oxidative DNA damage in a dose- and time dependent manner in A549 cells. On the other hand, recent study by Hanley[7] found that ZnO nanoparticles have intrinsic properties that enable them to preferentially kill cancerous T-cells while sparing healthy normal cells. The preferential toxicity of ZnO nanoparticles towards cancerous T-cells is of substantial magnitude (~28–35 times), especially in comparison with *ex vivo* indices of ≤10 reported for other commonly used chemotherapeutic agents using similar assays. This inherent differential toxicity of ZnO nanoparticles against rapidly dividing cancer cells raises exciting opportunities for their potential use as anti-cancer agents.

4. Conclusion

(1) Both the L929 cell and Hela cell proliferative activity were strongly deteriorated by different dose of 20nm ZnO nanoparticle suspension at 24h and 48h culture.
(2) The percentage of necrotic L929/Hela cell and the apoptotic L929/Hela cell are absolute majority in AnnexinV-FITC/PI-FCM assay.
(3) Feeding the ZnO nanoparticle suspension through digestive tract at a dose of 0.6mg daily led to the damage to some primary organs (heart, lung, liver and kidney) of mice.

(4) Sarcoplasmic reticulum expanded and organelle vacuolated in the mice heart, liver and brain, after feeding the ZnO nanoparticle suspension through digestive tract.

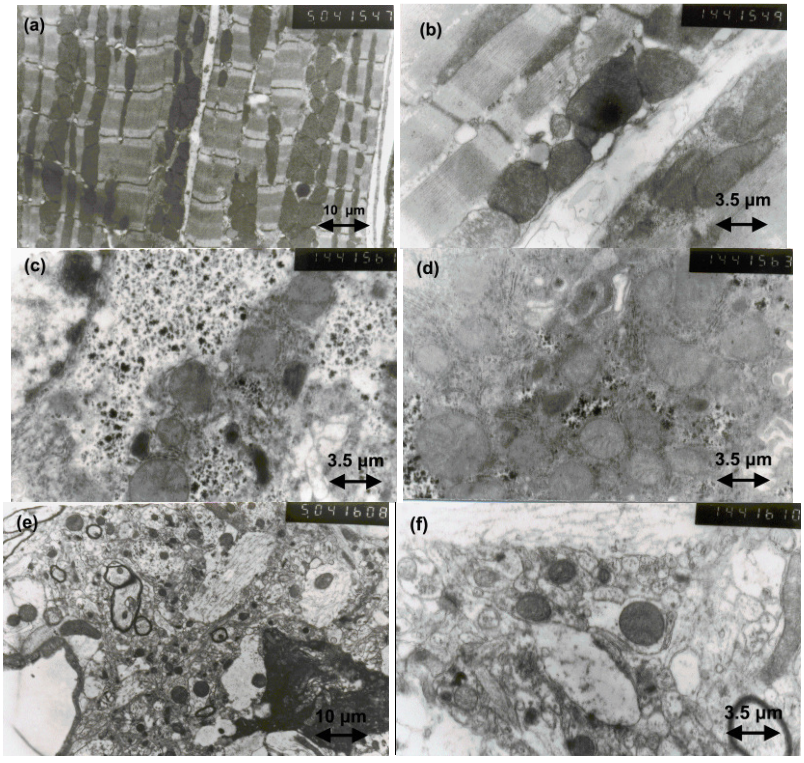

Fig. 6. TEM images of the mouse cardiac tissue(a)-(b), liver tissue(c)-(d) and brain tissue(e)-(f) after the 30mg/ml nano ZnO suspension was administered at a dose of 0.2ml through digestive tract, each time a day and lasted 7 days, then executed at the day 17.

References

1. Z. W. Pan, Z. R. Dai, Z. L. Wang, *Science* **291**, 1947 (2001).
2. Z. L. Wang, X. Y. Kong, Y. Ding, P. X. Gao, W. Hughes, R. S. Yang, Y. Zhang, *Adv. Funct. Mater.* **14**, 944 (2004).
3. J. Zhou, N. S. Xu, Z. L. Wang, *Adv. Mater.* **18**, 2432, (2006).
4. W. S. Beckett, D. F. Chalupa, A. Pauly-Brown, D. M. Speers, J. C. Stewart, M. W. Frampton, M. J. Utell, Li-Shan Huang, C. Cox, W. Zareba and G. Oberdörster, *American Journal of Respiratory and Critical Care Medicine* **171**, 1129 (2005).
5. F. Dechsakulthorn, A. Hayes, S. Bakand, L. Joeng and C. Winder, in *Proc. 6th World Congress on Alternatives & Animal Use in the Life Sciences*, August 21-25, 2007, Tokyo, Japan, AATEX 14, Special Issue, pp. 397-400.
6. W. S. Lin, Y. Xu, C. C. Huang, Y. F. Ma, K. B. Shannon, D. R. Chen, Y. W. Huang, *J. Nanopart. Res.,* (2008) DOI 10.1007/s11051-008-9419-7.
7. C. Hanley, J. Layne, A. Punnoose, K. M. Reddy, I. Coombs, A. Coombs, K. Feris and D. Wingett, *Nanotechnology* **19**, 295103 (2008).