# Nano-Safety Science for ensuring safety of nanomaterials ~Biological assessment of silica nanoparticles focused on neutrophil~

Kazuma Higashisaka[1,2], Akiyoshi Kunieda[1], Yuki Iwahara[1], Kota Tanaka[1], Shin-ichi Tsunoda[2,3], Yasuo Yoshioka[1,2], and Yasuo Tsutsumi[1,3]

[1]Laboratory of Toxicology and Safety Science, Graduate School of Pharmaceutical Sciences, Osaka University, 1-6 Yamadaoka, Suita, Osaka 565-0871, Japan., higashisaka@phs.osaka-u.ac.jp
[2]Laboratory of Biopharmaceutical Research, National Institute of Biomedical Innovation, 7-6-8, Saito-Asagi, Ibaraki, Osaka 567-0085, Japan.
[3]The Center for Advanced Medical Engineering and Informatics, Osaka University, 1-6, Yamadaoka, Suita, Osaka 565-0871, Japan.

## Abstract

Nanomaterials display unique properties compared to conventional materials. On the other hand, the debate on safety of nanomaterial has expanded worldwide. Therefore, it is essential to obtain more information to ensure the safety of nanomaterial. In this regard, it has been focused on neutrophil being involved in some biological responses. However, their detail information about the involvement with neutrophil and nanomaterial-induced biological responses is still little. Here, we examined the changes of neutrophil proportion induced by silica nanoparticles and attempted to gather basic information about the association with thier biological effects. BALB/c mice were treated intravenously of silica particles with diameters of 70 nm (nSP70) or saline. Our results showed that nSP70 would induce neutrophilia and it depends on nSP70-induced G-CSF elevation. We are currently analyzing the association with some adverse biological effects or kinetics after nSP70 exposure.

***Keywords:*** nanomaterial, neutrophil, G-CSF, dsDNA

## 1. Background

Nanomaterials with particle sizes below 100 nm display unique properties compared to conventional materials with a submicron size. Various types of nanomaterial have been designed and produced for consumer and industrial applications. On the other hand, the debate on safety of nanomaterial has expanded worldwide. In our previous study, we showed that silica nanoparticles with diameter smaller than 100 nm were more likely to cause consumptive coagulopathy [1] and pregnancy complications [2] in mice compared to silica particles with > 100 nm diameter. Therefore, to fully utilize the potential benefits of nanomaterial, it is essential to obtain more information to ensure the safety of nanomaterial. In this regard, it has been focused on neutrophil being involved in some biological responses such as *in vivo* kinetics and inflammatory responses after exposure to nanomaterial. However, their detail information about the involvement with neutrophil and nanomaterial-induced biological responses is still little. Here, we examined the changes of neutrophil proportion induced by silica nanoparticles, widely used in many consumer products such as cosmetics, food, and

medicine, and attempted to gather basic information about the association with silica nanoparticles-induced biological effects.

## 2. Materials and Methods

### 2.1. Silica particles

Silica particles were purchased from Micromod Partikeltechnologie (Rostock/Warnemünde, Germany). The silica particles with diameters of 70, 300, and 1000 nm (nSP70, nSP300 and mSP1000, respectively) were used in this study. They were sonicated for 5 min and vortexed for 1 min prior to use.

### 2.2 Experimental protocols

BALB/c mice were treated intravenously with 0.8 mg/mouse of nSP70, nSP300, and mSP1000 or saline. At 24 h after injection, the proportion of neutrophil in peripheral blood was evaluated by flow cytometry analysis.

### 2.3. In vivo depletion

Neutrophil depletion was achieved by injecting anti-Ly-6G antibody (clone 1A8; BioLegend, San Diego, CA) or isotype controls (clone RTK2758; BioLegend) intraperitoneally into BALB/c mice (n = 5 or 6 per group) 24 h prior to nSP70 injection. G-CSF depletion was achieved by injecting 50 μg/mouse of anti-G-CSF antibody (clone 67604; R&D systems, Minneapolis, MN) or isotype controls (clone 43414; R&D systems) intraperitoneally into BALB/c mice (n = 5 or 6 per group) 4 h prior to nSP70 injection.

## 3. Results and Discussion

### 3.1. Analysis of neutrophil proportion and activation in silica particles treated mice.

Initially, to evaluate the proportion of neutrophil in peripheral blood after treatment with silica particles, the mice were intravenously treated with nSP70, nSP300, or mSP1000. The results showed that the proportion of neutrophil in nSP70-treated mice resulted in significant increment at 24 h after treatment though those in nSP300- and mSP1000- treated mice showed no significant changes. Furthermore, this increment induced by nSP70 occurred in dose-dependent manner (Figure A). Myeloperoxidase (MPO) is one of the most abundant proteins in neutrophils and released when neutrophils are stimulated. Then, we evaluated the plasma level of MPO in silica particles- treated mice by ELISA. As with the result of proportion of neutrophil, higher plasma levels of MPO were observed only in nSP70-treated mice compared to that of saline-treated mice (Figure B). In addition, treatment of anti-Ly-6G antibody, specific for neutrophil, tended to suppress the nSP70-induced MPO production. These results indicated that nSP70 would induce neutrophil activation with an increase in proportion of neutrophil.

### 3.2. Granulocyte-Colony Stimulated Factor (G-CSF) expression in silica particle treated mice.

Next, to clarify the mechanism of nSP70-induced neutrophilia, we analyzed the expression of granulocyte colony-stimulating factor (G-CSF), which plays essential roles in proliferation or differentiation of neutrophil, in mice treated with each silica particles. The

mice were intravenously treated with nSP70, nSP300, or mSP1000 and the plasma level of G-CSF in each mouse was measured by ELISA. The results showed that the proportion of neutrophil in nSP70-treated mice resulted in significant increment at 24 h after treatment though those in nSP300- and mSP1000- treated mice showed no significant changes. Moreover, anti-G-CSF antibody-treated mice exhibited a significant decrease in neutrophil proportion. These results indicated that increasing neutrophil proportion induced by nSP70 depends on nSP70-induced G-CSF elevation. We are currently analyzing association between nSP70-induced neutropilia and some adverse biological effects or kinetics after nSP70 exposure. In the future, our study would contribute to develop the safety and efficacy nanomaterials.

## References


[1] Nabeshi H, Amorphous nanosilicas induce consumptive coagulopathy after systemic exposure., Nanotechnology, 23(4): 045101, 2012

[2] Yamashita K, Silica and titanium dioxide nanoparticles cause pregnancy complications in mice., Nat Nanotechnol, 6(5): 321-8, 2011


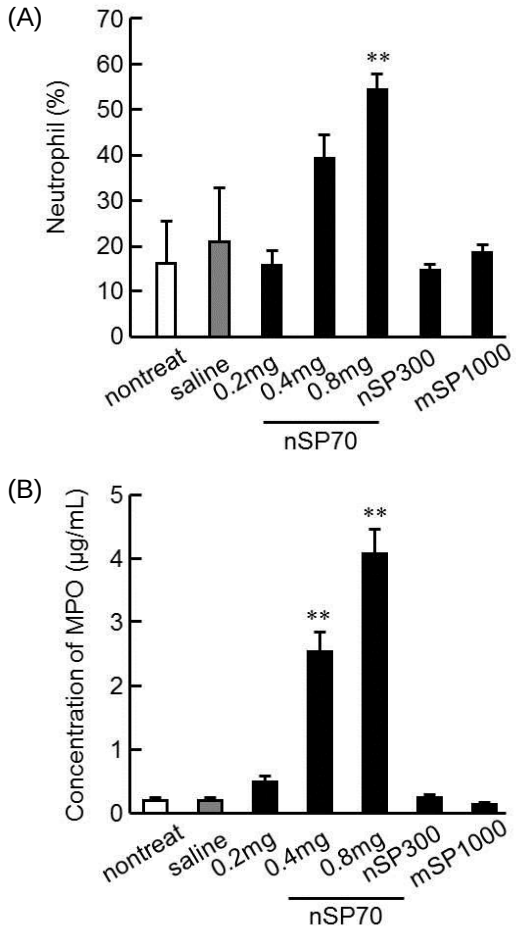


Figure: BALB/c mice were intravenously treated with nSP70, nSP300, or mSP1000 at 0.8 mg/mouse. After 24 h, (A) the proportion of neutrophil in peripheral blood of each mouse was analyzed by flow cytometry and (B) the level of MPO in plasma of each mouse were measured by ELISA. Data are presented as mean ± S.E.M. (n = 5; $*P < 0.05$, $**P < 0.01$ *versus* value for saline-treated group by ANOVA).