Investigation on genotoxic-oxidative effects of SiO_2 50 nm exposure: comparison between in vitro study and in vivo results on exposed workers

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INTRODUCTION

The increasing use of SiO_2 nanoparticles (NPs) in several sectors such as cosmetics, food and rubber reinforcement raises the question of the exposure of workers by inhalation, that is the most important route for nanomaterials. Nanomaterials, including SiO_2NPs, can stimulate inflammatory responses and induce oxidative effects and DNA damage, therefore the main aim of this study was to evaluate the genotoxic and oxidative effects of exposure to SiO_2NPs 50 nm on human bronchial cells.

Final purpose was to compare the obtained *in vitro* results with those previously found by the biomonitoring of workers who were producing the same NPs.

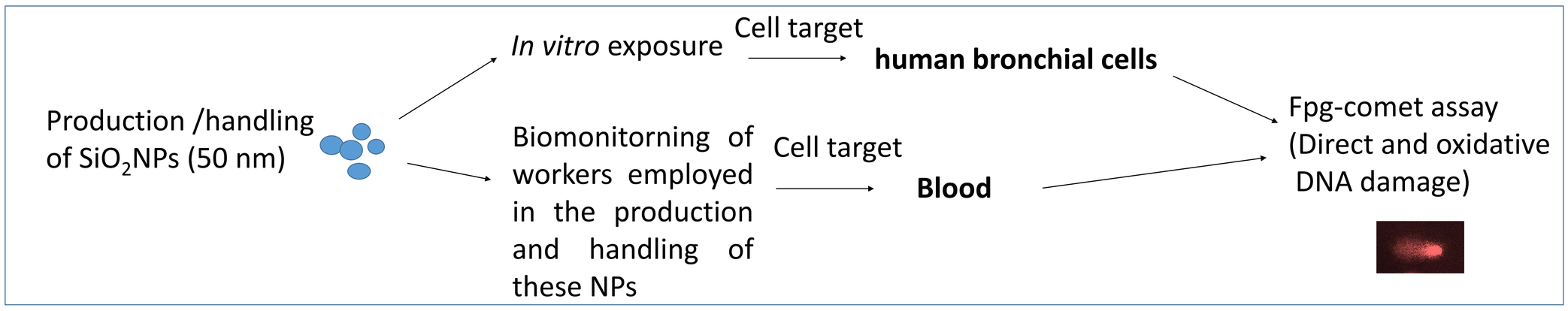


Figure 1. *Study design to evaluate early genotoxic effects of occupational exposure to SiO_2 NPs by the comparison of in vitro results with those obtained on workers who produced the same NPs*

MATERIALS AND METHODS

Cell line cultures

As in vitro target, we used human bronchial BEAS-2B cells (ATCC) cultured in bronchial epithelial cell growth medium (BEGM) supplemented with human epidermal growth factors according to manufacturer's instructions (Lonza, Belgium). The cells ($8x10^4$ cells/well) were seeded into 24 multi-well culture plate and cultured for 48 hours before the exposure. Semiconfluent cell cultures were exposed for 24 hours to the concentrations of 2, 10 and 40 µg/ml for 24h.

Dispersion preparation and characterization

The tested SiO_2 NPs, synthesized by precipitation, were furnished by HiQ nano (Lecce, Italy). A stock solution of 1 mg/ml was prepared suspending NPs in ultrapure sterile water, vortexed 1 min and then sonicated 30 min by probe sonicator (Heat Systems Misonix, 550W, 20 kHZ, amplitude 15 %, probe sonicator 13 mm).

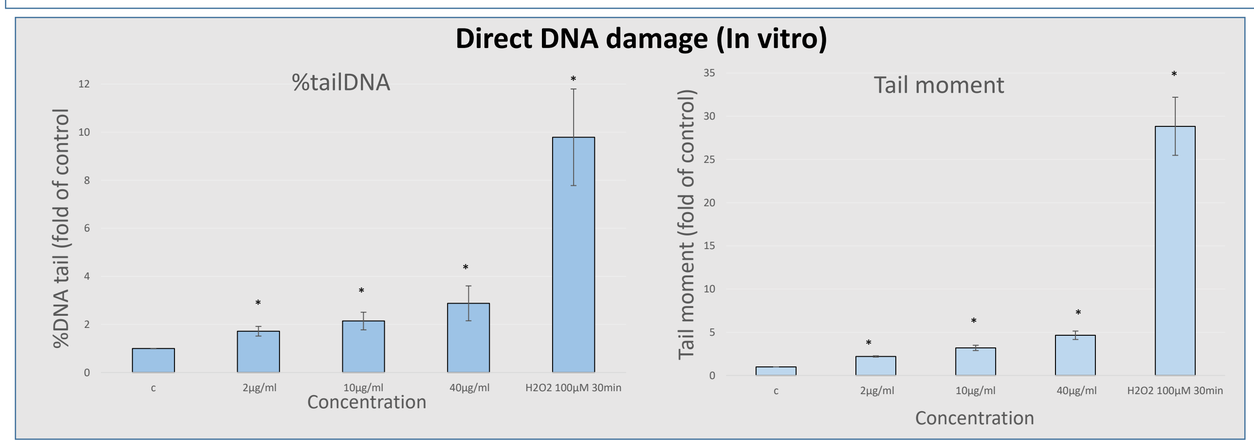
The hydrodynamic diameter of SiO_2NPs suspensions were measured by Dynamic and Electrophoretic Light Scattering (DLS and ELS) analysis using a Zetasizer nano ZS (Malvern, UK).

Biomonitoring

As target of the biomonitoring we used lymphocytes of 6 exposed workers and 6 unexposed subjects. We evaluated direct and oxidative DNA damage by fpg-comet assay using tail DNA% and tail moment (TM) comet parameters.

Fpg-modified comet assay

Direct/oxidative DNA damage was evaluated by Fpg-modified comet assay according Collins protocol (Collins et al. 1993) with minor modifications. Unexposed cells were used as negative control and cells exposed for 30 min to 100 µM H_2O_2 were used as positive control. Images of 100 randomly selected comets either from Fpg enzyme treated or untreated slides, stained with ethidium bromide were acquired and analyzed from each sample, with specific image analyzer software (Delta Sistemi, Rome, Italy). The percentage of DNA in the tail *(%tailDNA*) and tail moment (*TM*) were obtained from the analysis. For each experimental point we calculated the mean values of such parameters of comets from enzyme untreated cells (*%tailDNA* and *TM*) which indicate the direct DNA damage, and those of comets from Fpg-enzyme treated cells (*%tailDNAenz* and *TMenz*), evaluating direct and oxidative DNA damage. Direct DNA damage was assessed calculating, for each experimental point, the values of exposed cells normalized in respect to unexposed cells (ratio *%tailDNA* and *TM* values of exposed cells vs *%tailDNA* and *TM* values of control cells respectively). Oxidative DNA damage was evaluated in terms of oxidized DNA bases (sites recognized and cut by Fpg) and calculated deducting *%tailDNA* and *TM* from the *%tailDNAenz and TMenz*, both in exposed and unexposed cells.



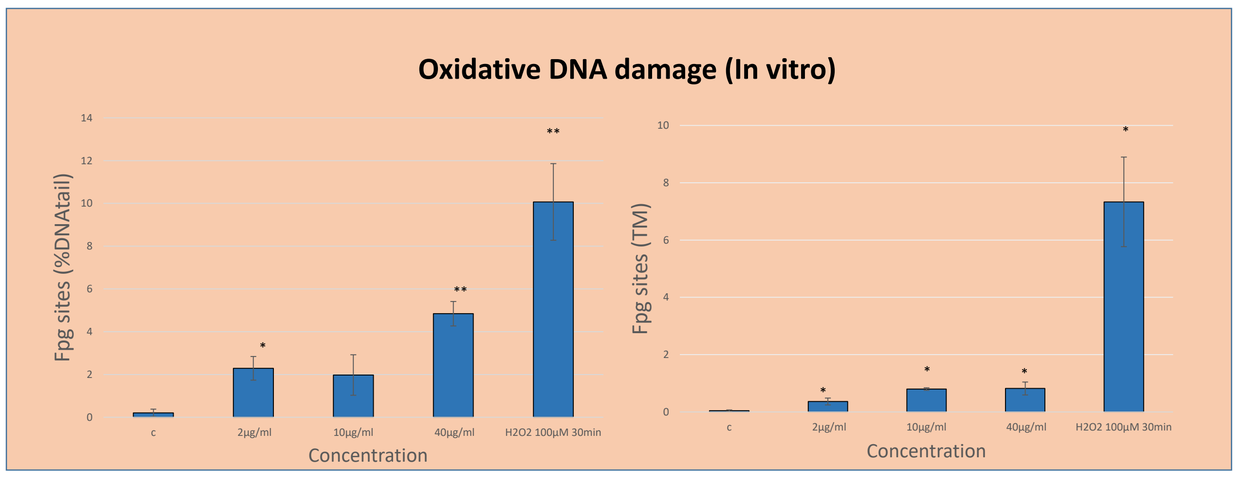


Figure 2. Direct (upper panels) and oxidative (lower panels) DNA damage evaluated by fpg-comet assay on BEAS-2B cells exposed for 24 hours to SiO_2 NPs. Data represent the means of three independent experiments. Cells exposed for 30 min to 100 µM H_2O_2 were used as positive control.* $p \leq 0.05$.

RESULTS

DLS analysis of the stock solution showed that Z-average diameter mean value was 216 nm with a PDI 0.416.

On the exposed cells we found a dose-dependent significant increase of direct DNA damage in terms of tail DNA% and TM and induction of oxidative DNA damage vs unexposed cells (figure 2).

Our previous biomonitoring study (Ursini et al. 2021), performed on workers employed in the production and handling of the same NPs that we tested *in vitro,* demonstrated on exposed workers higher values of both comet parameters compared to controls, with 17.22 vs 11.20 for *%tailDNA* and 5.69 vs 3.80 for TM.

The comparison of the two studies (in vitro and in vivo on exposed workers) confirmed the potential genotoxicity of the tested/handled SiO_2 nanopaticles but not oxidative effect induction. It could be explained by the different cell type and/or by the involvment of in vivo repair mechanisms.

DISCUSSION AND CONCLUSIONS

The results of this study that used two different exposure scenarii strategies, demonstrate in different targets, lung (*in vitro*) and blood (*in vivo*), the same kind of effect due to SiO_2 NPs exposure. These findings suggest that the used experimental *in vitro* model is useful for nanosafety screening and could be used to predict the possible genotoxicity of SiO_2 NPs on workers exposed during the production process.

REFERENCES

- Collins, A.R., Duthie, S.J., Dobson, V.L., 1993. Direct enzymic detection of endogenous oxidative base damage in human lymphocyte DNA. *Carcinogenesis*, 14 (9), 1733-1735.
- Ursini CL et al. 2021 Occupational exposure to graphene and silica nanoparticles. Part II: pilot study to identify a panel of sensitive biomarkers of genotoxic, oxidative and inflammatory effects on suitable biological matrices. *Nanotoxicology* Mar;15(2):223-237. 2021.