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Methodological approaches influencing cellular uptake and cyto-(geno)toxic effects of nanoparticles.

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1. Introduction

Cytotoxicity and genotoxicity of nanoparticles (NPs) has gained a lot of interest in the last decade, evidenced by the increasing number of studies in this field. The applicability of the current tests has been questioned and some efforts have been made to review the assays for their adequacy for NP testing [1,2]. Here the influence of serum and the use of cytochalasin-B is assessed on the cellular uptake of amorphous silica NPs (SNPs) and their biological effect.

Keywords: Serum, *in vitro* cytochalasin – B micronucleus assay, amorphous silica nanoparticles, genotoxicity

2. Materials and Methods

Amorphous silica nanoparticles were synthesized and characterized as described in Thomassen *et al.* (2009) [3]. 12 nm lysine-SNP and 16 (S-16) and 60 nm (S-60) Stöber SNPs were used. A549 lung carcinoma cells were treated with the SNPs in presence (10%) or absence (0%) of fetal bovine serum. Either a MTT assay was performed after 24h or nuclearity was scored after 40h. In the latter case 4h after treatment, 2.5 µg/ml cytochalasin-B was added to the culture. Inductively-coupled plasma mass spectrometry was performed to quantify the cellular Si content in A549 cells, after different treatment.

3. Results and Discussion

Cytotoxicity of NPs: A question of serum?

To assess what the influence of serum is when considering the cytotoxicity of SNPs, a tetrazolium salt-based cytotoxicity assay (MTT assay) was performed, in presence (10%) and absence of fetal bovine serum during 24h. Previously interference of the SNPs with the assay was excluded. Results indicate that in absence of serum, SNPs induce 50% cytotoxicity at approximately 10-20 times lower concentrations, compared to treatment conditions where 10% serum was present (Figure 1).

This was confirmed for another type of amorphous SNP (diameter 12nm), differing in synthesis method, i.e. lysine-silica, with a different approach. Microscopy analysis shows normal cell morphology and cell growth under control conditions in presence and absence of serum and when cells are treated with 10µg/ml SNPs (d=12 nm) during 40h in presence of serum. Cultures treated with 10µg/ml SNPs under serum starvation conditions exhibit cytotoxicity and strongly reduced cell division rates (Figure 2).

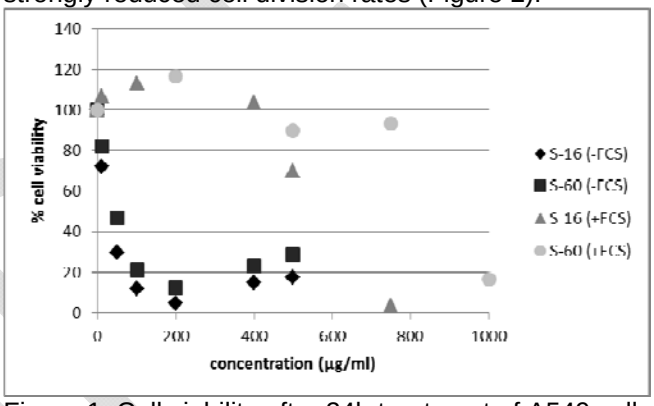

Figure 1: Cell viability after 24h treatment of A549 cells with 16 nm (S-16) and 60 nm (S-60) SNPs in presence and absence of fetal calf serum (FCS).

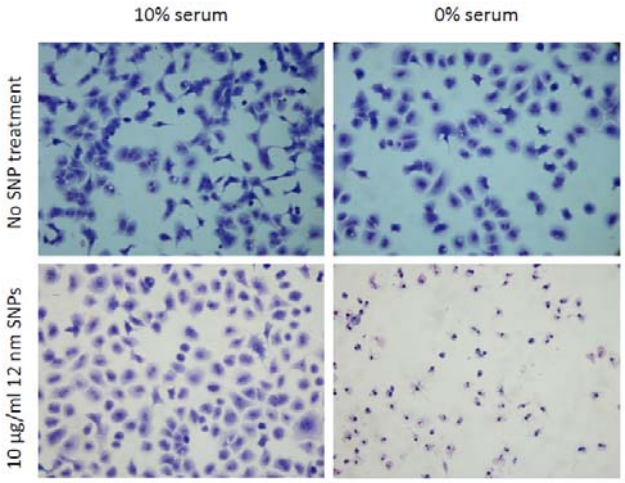

Figure 2: Pictures with 400x magnification under Leica microscope after 40h culture in presence (left panels) and absence (right panels) of fetal calf serum. The lower panels represent cultures treated for 40h with 10µg/ml SNPs (12 nm).

Cytochalasin-B treatment allowed quantification of the nuclearity and revealed a strong decrease in cell division after treatment with concentrations of 5 and 10 µg/ml SNPs in absence of serum (Figure 3).

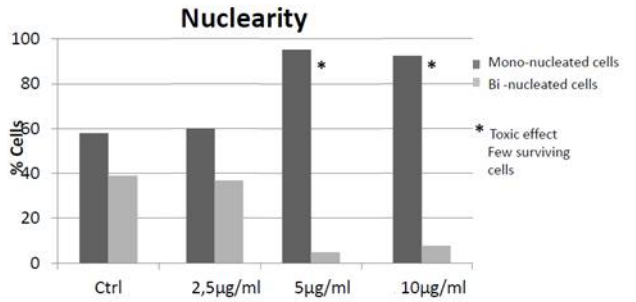

Figure 3: Nuclearity after 40h of treatment with 12 nm SNPs in absence of serum.

These results indicate that the use of serum has an influence on the read-out of cytotoxicity assays. Therefore attention should be paid when interpreting cytotoxicity results as serum starvation conditions are maybe less physiologically relevant for hazard identification.

Treatment of A549 cells with fluorescent SNPs showed a different cellular uptake depending on the serum percentage used (data not shown).

Serum might have an impact on the outcome of the measured endpoint in two ways:

1. The toxicity of the SNPs is decreased by binding of serum proteins on the surface of the SNPs.
2. The toxicity of SNPs is decreased because of a reduction in cellular uptake of the particles.

In vitro cytochalasin-B micronucleus assay

The *in vitro* micronucleus assay allows the detection of both clastogenic and aneugenic events. Addition of cytochalasin-B enables identification of cells that divided. Cell division is required for the expression of micronuclei. Cytochalasin-B is an actin inhibitor and hence can interfere with the cellular uptake. Here we quantified the cellular uptake of 16 and 60 nm SNPs by ICP-MS when cytochalasin-B is added at different timepoints and under cytochalasin-B-free conditions (Figure4).

Results show an increasing uptake of SNPs over time reaching a plateau at 6 – 24h. The use of cytochalasin-B generally lowers the cellular Si content in the cells as measured by ICP-MS (Figure 4).

Our results indicate that some methodological approaches such as the use of serum and cytochalasin-B in assays may modulate the outcome of the assay by decreasing the cellular uptake of the SNPs. Therefore the experimental design and choice of the assay are of great importance, as well as a correct interpretation of the data.

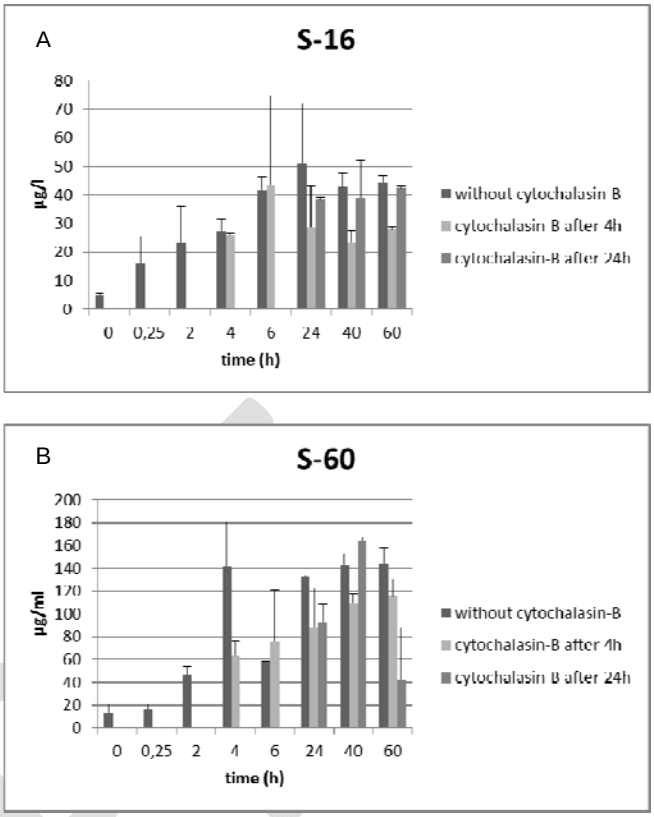

Figure 4: Quantification of the cellular Si content after different treatment times with 16 nm (S-16) and 60 nm (S-60) SNPs. Cellular Si content was also measured under conditions where cytochalasin-B was added 4h or 24h after treatment.

Acknowledgments

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References

[1] S.H. Doak, S.M. Griffiths, B. Manshian, N. Singh, P.M. Williams, A.P. Brown, G.J. Jenkins. Confounding experimental considerations in nanogenotoxicology. Mutagenesis 4: 285-293 (2009)

[2] L. Gonzalez, BJS Sanderson, M. Kirsch-Volders. Adaptations of the in vitro MN assay for the genotoxicity assessment of nanomaterials. Mutagenesis 26:185-191 (2011)

[3] L.C Thomassen, A. Aerts, V. Rabolli, D. Lison, L. Gonzalez, M. Kirsch-Volders, D. Napierska, P.H. Hoet, C.E. Kirschhock, J.A. Martens. Synthesis and characterization of stable monodisperse silica nanoparticle sols for in vitro cytotoxicity testing. Langmuir 26(1):328-35 (2010).