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What is the impact of Silicon Carbide nanoparticles to the mineral composition of rat lungs? A PIXE-μPIXE comparative study

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Abstract. The exposure to nanomaterials can yield changes in the mineral composition of tissues which may have long term health repercussions. In this study, the changes in mineral composition of rat lungs, exposed to a nanoaerosol of silicon carbide (SiC), has been studied by means of global and local ion beam probes with the Particle-Induced X-ray Emission (PIXE) technique, measuring the whole lung contents and selected areas where SiC was found, respectively. It was found that from a global perspective there is a small decrease in the mineral contents (phosphorous, sulphur, chlorine and potassium) of the lung except for Ca, while locally these mineral contents tend fluctuate.

1. Introduction

Nanosafety and nanotoxicology studies focus mainly in the impact of nanomaterials (NMs) through the analysis of biomarkers and, when possible, the quantification of the NM dose within the target such as tissues or cells [1-3]. One aspect often overlooked in these studies is the fact that the presence of an external agent in the media environment can cause alterations to the same media which may result in an increase or decrease of the media contents, such as proteins or minerals, and hence have an impact on the composition of the target. For example, recent *in vivo* studies with silicon carbide (SiC) and titanium carbide (TiC) NMs in oral administration have shown a perturbation of the mineral absorption within the gastrointestinal tract [1, 4].

In this study the impact of a SiC nanoaerosol on the composition of rat lungs is investigated. SiC was selected as a model NM due to its extensive industrial usage [5]. The mineral composition of rat lungs exposed to SiC was quantified by Particle-Induced X-ray Emission (PIXE) from two perspectives: using a broad beam (PIXE analysis) to quantify the lung overall mineral contents, and using a micro beam (μPIXE analysis) to quantify the lung mineral contents in areas around SiC deposition.

2. Materials and methods

2.1. Animals

Nulliparous and non-pregnant female rat Sprague-Dawley (8 weeks, 190-200 g; supplier: Charles River Laboratories) were selected for this study. Each one was housed in ventilated cages and was acclimatized 2 weeks previous to the exposure. Experiments were performed with the agreement of the Committee on the Ethics of Animal Experiments of the University of Namur (protocol "FUNDP10/128 DO inhalation", approved in April 2010).

Rats were sacrificed at 0, 3, 7, or 28 days after exposure, and their lungs were excised after a heart lavage.

2.2. Exposure

Silicon carbide (SiC) NPs were bought from Io-Li-Tec. No trace of endotoxins was found and it was used without further treatment for nanoaerosol production.

Rats were exposed in a Whole-Body Exposure Model based on the Organisation for Economic Co-operation and Development (OECD) guideline 403, as described by Laloy et al. [6]. Rats were exposed 6 h a day during 5 consecutive days to a SiC nanoaerosol diluted at $2.5x10^5$ particles/cm^3. The control group was exposed to the same filtered air used to produce the nanoaerosol.

2.3. Sample preparation

For broad beam measurement, rat lungs were dried 48 hours at 37 °C and prepared into pellets following a well-established protocol for high volume analysis [2].

For micro beam measurement, rat lungs were frozen in liquid nitrogen and sections of 6 x 6 mm^2 and 10 µm thick were obtained with a cryostat microtome. The sections were placed on polypropylene films fixed to aluminium holders and were left to dry at ambient conditions without any further treatment.

2.4. Ion beam analysis

Samples were chemically quantified with a broad beam (few mm^2) at the Laboratory of Analyses by Nuclear Reaction (LARN) of the Physics of Matter and Radiation Unit of the University of Namur, and with a micro beam (few μm^2) at the Surrey Ion Beam Centre. The Particle-Induced X-ray Emission (PIXE) and Rutherford Back-Scattering (RBS) techniques were used to measure heavy elements (i.e. Si & Ca) and light elements (i.e. C & O), respectively. The principles of PIXE and RBS have been described elsewhere [2]. The techniques PIXE and RBS are called as such for broad beam analysis, while the "µ" prefix is added for micro beam analysis, hence µPIXE and µRBS. RBS (µRBS) data was used to adjust the matrix for PIXE (µPIXE) analysis. The measurement geometry for broad beam has been described previously [1], and used a 2.0 or 2.5 MeV proton beam in this study. The geometry for the micro beam has been described previously [7], and used a 2.5 MeV proton beam in this study.

2.5. Statistical analysis

Data was analyzed using the Holm–Sidak method (two way ANOVA). The statistical significance was compared between the control and exposed samples, dividing the significant results in $p<0.05$, $p<0.01$, and $p<0.001$.

Three samples (n=3) per exposure condition were measured with broad beam. Eight (n=8) scans were done per exposure condition and four (n=4) scans were done per control condition were measured with micro beam.

Mineral concentrations are presented as the weighted mean concentration with error bars representing 1 standard deviation of the weighted mean. Ratios between exposed and control samples use an error bar taking into account the propagation of errors from both samples.

3. Results and discussion

The mineral concentration found in the rat lungs is presented in Table 1 for PIXE measurements and Table 2 for µPIXE measurements. In the case of PIXE measurement the mineral concentration change is easier to observe for rats sacrificed at day 28: phosphorous (P), sulfur (S), chloride (Cl) and potassium (K) show a lower content with respect to their respective controls; while Ca showed a statistically significant increase with respect to the control. The results are summarized in Figure 1a.

Table 1. Elemental analysis of trace minerals in lungs measured by PIXE. The second column gives the dry lung weight. Statistically significance exposed versus control group: * indicates p<0.05, * indicates p<0.001. MDL: Minimum level of detection.**

Controlgroup Sacrifice day	Weight (mg, mean ± SD)	Elemental analysis (wt. ppm, weighted mean ± SD)					
		Si	P	S	Cl	K	Ca
0	183 ± 22	< MDL	21,368 ± 469	13,021 ± 514	20,471 ± 1,141	15,825 ± 704	601 ± 58
3	190 ± 37	< MDL	21,118 ± 620	12,701 ± 487	27,027 ± 5,909	16,033 ± 1,350	621 ± 97
7	220 ± 24	< MDL	22,822 ± 885	14,578 ± 429	20,813 ± 917	17,928 ± 656	680 ± 68
28	212 ± 14	< MDL	25,319 ± 2,183	15,632 ± 967	25,456 ± 3,698	17,184 ± 1,472	851 ± 106
Exposed group Sacrifice day		Si	P	S	Cl	K	Ca
0	233 ± 32	5,039 ± 789 ***	20,330 ± 281	12,745 ± 440	23,059 ± 4,367	14,206 ± 1,686	654 ± 101
3	209 ± 17	5,216 ± 867 ***	20,231 ± 633	12,865 ± 534	16,742 ± 1,090	16,592 ± 863	553 ± 89
7	166 ± 27	3,766 ± 207 ***	21,685 ± 1,611	13,398 ± 1,092	22,497 ± 2,923	16,882 ± 1,951	667 ± 26
28	291 ± 96	40 ± 10 *	20,048 ± 3,348	12,163 ± 2,020	17,623 ± 3,522	14,364 ± 3,266	1,264 ± 251 *

Table 2. **Elemental analysis of trace minerals in lungs measured by µPIXE. The second column gives the dry lung weight. Statistically significance exposed versus control group: * indicates p<0.05, *** indicates p<0.001.**

Control group Sacrifice day	Weight (mg, mean ± SD)	Elemental analysis (wt. ppm, weighted mean ± SD)					
		Si	P	S	Cl	K	Ca
0	183 ± 22	188 ± 29	10,092 ± 450	6,400 ± 293	9,630 ± 498	9,919 ± 476	211 ± 9
3	190 ± 37	174 ± 61	7,419 ± 84	4,679 ± 83	8,014 ± 172	7,498 ± 85	147 ± 8
7	220 ± 24	104 ± 10	7,067 ± 293	5,088 ± 210	5,701 ± 232	7,401 ± 315	241 ± 12
28	212 ± 14	82 ± 9	6,994 ± 155	4,391 ± 63	6,724 ± 114	6,826 ± 118	142 ± 10
Exposed group							

Sacrifice day		Si	P	S	Cl	K	Ca
0	233 ± 32	4,393 ± 143 ***	9,603 ± 224	5,254 ± 143 *	9,041 ± 335	9,429 ± 235	220 ± 15
3	209 ± 17	3,569 ± 164 ***	8,215 ± 243	5,064 ± 148	7,542 ± 284	7,995 ± 240	209 ± 4
7	166 ± 27	1,903 ± 220 ***	5,210 ± 468 *	3,282 ± 272 *	22,146 ± 3,317 *	4,043 ± 467 *	397 ± 163
28	291 ± 96	732 ± 33 ***	8,520 ± 155 *	5,787 ± 104 *	9,001 ± 198	8,282 ± 140 *	224 ± 10

Mineral concentration by µPIXE showed a marked, and in some cases a statistically significant, increase in all elements with respect to the controls for days 3 and 28; with a marked decrease for P, S and K and a marked increase in Cl and Ca at day 7. The results are summarized in Figure 1b. Of the measured elements, Cl presents the highest errors bars and it is also known as a common contaminant elements for PIXE, hence its results may not be representative without further detailed measurements.

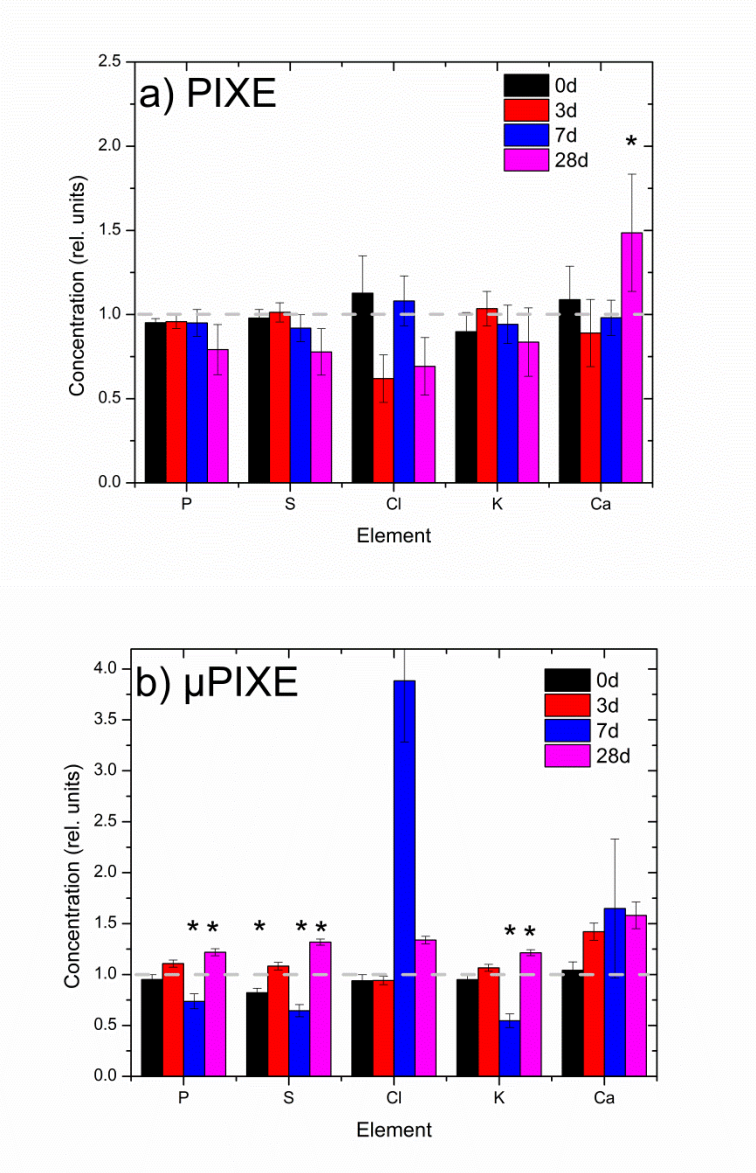

Figure 1. Control normalized mineral concentration of rat lungs. Statistically significance exposed versus control group: * indicates p<0.05. See *Statistical analysis* subsection for details on the error bar calculation.

The study of mineral composition variations is motivated by extracting more knowledge about the possible changes to key minerals such as Ca and P, whose ions control many of the cellular processes [8, 9], or electrical impulse conduction from ions of K in the neuron function [10], the regulation of osmotic pressue and acid-base balance by Cl [11], and the role of disulfide bonds in protein assemble and structure [12].

There is a difference between the statistically significant mineral changes in terms of concentration and days found in PIXE and μPIXE measurements. This difference reflects the type of sample being measured and hence the point of view obtained by each type of measurement: with PIXE the measured sample represents the whole lung, pulverized and made into a pellet, and therefore it reflects a global value on the total lung mineral composition. Taking into account that SiC likely remains inside the lung as observed due to the lack of impact on plasma analysis and the low levels of induced inflammation [6], most of the mineral changes, if any, should occur in the lung alveolar sac and as such any mineral composition should be relatively less evident than in the vicinity of the NM localization. On the other hand, μPIXE measurements were done on sections where SiC was found and as such it reflects a local perspective of the exposure, hence reflecting more statistical significant changes in the mineral composition. Indeed this is the case for the mineral concentration in P, S and K. From both perspectives it is judged that locally the alveolar sacs, due to the limited inflammation process, experience a depletion of minerals (7 days) which then overcompensates with higher concentrations (28 days), while as a whole the lung is effectively experiencing a decrease in minerals.

4. Summary

The mineral concentration of rat lungs exposed to an acute SiC nanoaerosol was investigated by PIXE and studied from the global lung mineral composition and local lung sections exposed directly to SiC, using a broad beam and a micro beam, respectively. While globally there is a slight decrease in mineral composition, local lung sections show a fluctuation of minerals.

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