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Non-invasive biomarkers of early effects for long-term monitoring of workers employed in a nanomaterials research laboratory

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INTRODUCTION

Workers employed in materials science research involving nanomaterials (NMs) may be exposed, particularly during NM weighing phase to produce nanocomposites (including those for 3D printing), to different NMs such as Carbon Nanotubes (CNTs), graphene nanoplatelets (GNPs) and metal oxide nanoparticles (NPs). These activities are continuously evolving to develop new materials, with changes over time in the type of exposure. Suitable biological matrices and biomarkers of early effect are needed to monitor over time the potential risk of occupational exposure.

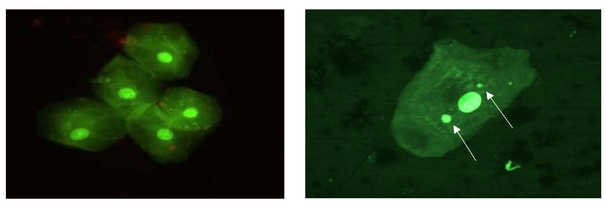

Fig. 1 BMCyt assay - *Exfoliated buccal cells stained with Acridine Orange. The arrow indicates the presence of a Micronucleus (MN).*

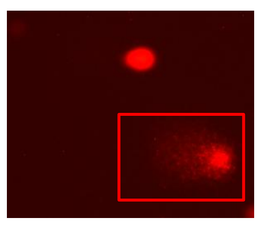

Fig. 2 *Fpg-Comet Assay - Exfoliated buccal cells stained with ethidium bromide. The box indicates a cell with damaged DNA (comet).*

MATERIALS AND METHODS

We enrolled 5 researchers of a research laboratory in material science producing nanocomposites (including those for 3D printing), potentially exposed to different NMs (such as CNTs, GNPs and metal oxide NPs) and 12 controls and performed environmental and biological monitoring, in two sampling campaigns carried out one year apart and characterized by different carbon-based production processes (GNPs and CNTs nanocomposite and polymer-matrix carbon nanocomposites for 3D printing, respectively).

Environmental exposure monitoring:

Harmonized OECD – ISO CEN methodology [1] was used to characterize workplace exposure by real-time measurements of particle number concentration (PNC) and average diameter (D_{avg}) of airborne nanomaterials, integrated with SEM-EDS analysis of particulate matter collected on filters by inertial impactors.

Biological monitoring:

Buccal Micronucleus Cytome (BMCyt) assay and Fpg-comet test on exfoliated buccal cells were used for local cyto-genotoxic and genotoxic/oxidative effects evaluation respectively. In particular, by Buccal Micronucleus Cytome (BMCyt) assay, we analysed the frequencies of cells with Micronuclei (MN) and Nuclear Buds (NB) for genotoxic effects, the frequencies of cells with Condensed Chromatin (CC) and of karyolitic (KL) and Binucleated (BIN) cells for cytotoxic effects (Fig.1). The percentage of subjects positive to MN assay (with frequency>cut off=1,5‰) was also calculated [2].

Fpg-comet test on exfoliated buccal cells (Fig.2) was used to detect direct DNA damage analysing comet parameters %DNA tail, Tail moment (Tm) and Tail length (TL) and oxidative DNA damage was estimated as the difference between %DNA tail in Fpg-treated (%DNA tail enz) and Fpg-untreated cells (%DNA tail); subjects with values >4 were considered positive to oxidative DNA damage [3]

RESULTS

Workplace monitoring

First monitoring: In the first monitoring, the mean value of PNC (range 10-700 nm) measured in the workers's personal breathing zone during the activities with CNTs outside the hood, was ~1.4 times the far-field background (2,600 part/cm^3; SD 400 part/cm^3), while during activities with GNPs it does not deviate from the background values. The corresponding mean diameter (D_{avg}) of airborne particles in the processing area (range 10-300 nm) was similar to the background far-field (office) and equal to approximately to 50 nm.

Second monitoring: In the second campaign, PNC during CNT weighing phase was higher than office/background (15,700 vs 9,500 part/cm^3) with D_{avg} 51 and 40 respectively.

Biological monitoring

First monitoring:

BMcyt and Fpg comet assay did not show in exposed subjects cyto-genotoxic or oxidative effects. (Table 1)

Second monitoring:

BMCyt assay showed increased cytotoxicity in exposed subjects in respect to controls (higher binucleated and reduced karyolitic cells frequencies).

The Fpg-comet assay showed direct and oxidative DNA damage (60% of oxidative DNA damage positive subjects vs 0% of the first sampling). (Table 1)

Table 1. Biomonitoring results

				BMcyt assay					Fpg-Comet assay			
	Age	Work seniority years	Smoking habit	MN‰ Pos>cutoff=1.5	NB ‰	CC ‰	BIN ‰	KL‰	%DNA Tail	Tm	TL	Oxidative DNA damage (%DNA Tail enz-%DNA Tail) (Pos>cut off=4)
I biomonitoring Exposed workers N=5 mean±SD	28.4±6.2	1.45±1.6	1Yes (20%) 4 No (80%)	0.76±0.98 Pos 1/5 (20%)	0.47±0.57	0.28±0.26	1.1±0.75	**41.13±18.8*** P=0.004	17.38±4.7	7.46±1.9	30.4±7.1	0.76±1.04 Pos 0/5 (0%)
II biomonitoring Exposed workers N=5 mean±SD	29.4±6.2	2.45±1.6		0.85±1.45 Pos 1/5 (20%)	0.74±1.05	0.19±0.43	**3.98±2.38*** p=0,03vs ct	**65.72±27.9*** P=0.039	**23±2,15*** P=0,007vs ct P=0,03 I vs II biomonit	7.03±1.4	27.9±4.8	4.78±3.53 Pos 3/5 (**60%**)
Controls N=12	34.6±6.3		4 yes (33%) 8 No (67%)	0.91±0.90 Pos 3/12 (25%)	0.38±0.41	0.28±0.49	2.42±0.47	95.1±36.1	19.47±2.51	6.74±1.1	29.9±8.4	3.46±3.0 Pos 3/12 (25%)

* T test p>0.05

CONCLUSIONS

The findings show induction of local cyto-genotoxic and oxidative effects associated with the second monitoring performed during the production of polymer-matrix CNT nanocomposites for 3D printing and characterized by higher exposure (PNC value) during CNT weighing. In particular, the increase in binucleated cells associated with a reduction in dead cells without nuclei (karyolytic cells) indicates an alteration of cell division (potential cytostatic effect) combined with a slowing down or blockage of the normal processes of programmed cell death (apoptosis/terminal differentiation). The presence of direct and in particular of oxidative DNA damage seems to be in agreement with the suggested genotoxic/oxidative effects of carbon-based nanomaterials exposure.

Although limited by the small number of subjects, the results of this multidisciplinary study support the suitability of used sensitive, non-invasive biomarkers to monitor over time workers involved in different nanomaterial production processes.

References

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