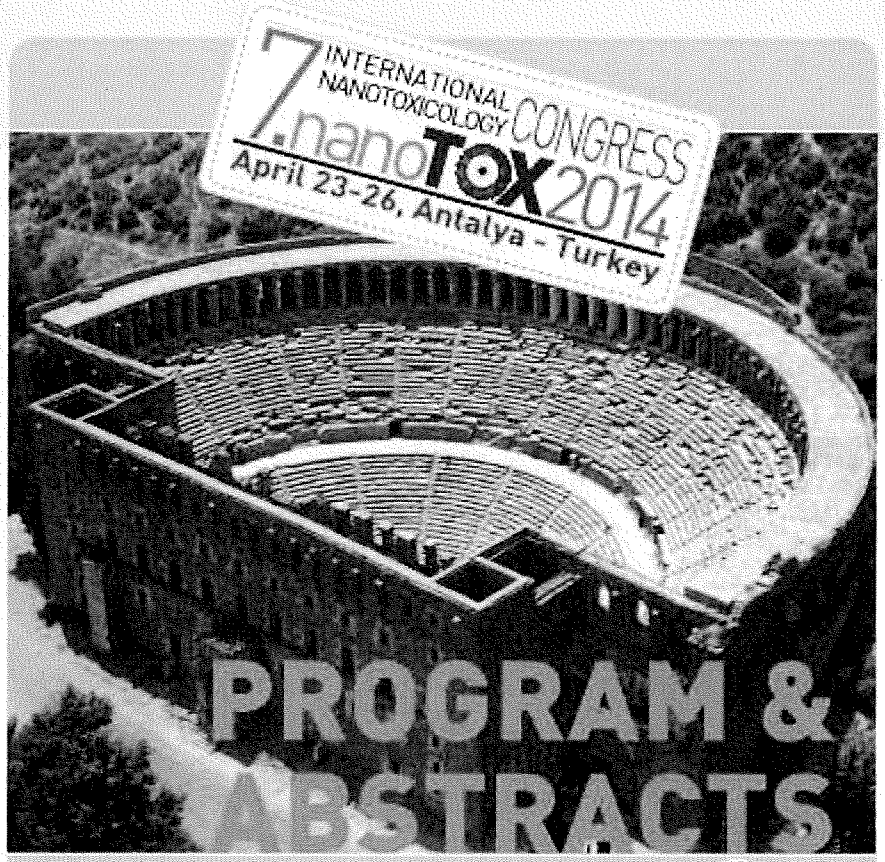

Under the auspices of the Turkish Society of Toxicology

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community, to date. Current approaches generally rely on the use of NMs that have a fluorescent or radioactive label to facilitate their detection and often involve complex sample preparation procedures. Accordingly, new approaches to visualise the uptake of NMs by cells and tissues using the inherent properties of (unmodified) NMs are urgently required. Coherent anti-Stokes Raman scattering (CARS) microscopy is based on the intrinsic physicochemical properties of matter and allows ultrasensitive detection in living and fixed cells and tissue with high chemical specificity, nm resolution and minimal sample preparation. Despite the advantage that unmodified NMs can be imaged using CARS microscopy it has only been used to a very limited extent to investigate NM uptake by cells. This study used gold (Au) and titanium dioxide (TiO2) NMs to test the suitability of CARS to image the uptake of different NMs by (live and fixed) cells and tissues obtained from in vitro and in vivo studies. J774 macrophage and C3A hepatocyte cell lines were exposed to NMs (15-31µg/ml) for up to 4 hours. Rats were exposed to NMs (62µg) via intratracheal instillation. A bronchoalveolar lavage (BAL) was performed, blood was taken (and white blood cells (wbcs) isolated) and lung and liver tissue fixed and sectioned. Fixed and live cell imaging via CARS confirmed that macrophages and hepatocytes were able to internalise TiO2 and Au NMs, and z stacks were used to confirm the uptake into the cell interior. It was evident that NM uptake by macrophages was more easily imaged when compared to hepatocytes, and thus some cell types may be more amenable to CARS microscopy than others. TiO2 uptake by alveolar macrophages and blood wbcs isolated from exposed rats was detected using CARS microscopy. In addition, TiO2 and Au NMs were imaged in lung and liver tissue of exposed rats. This study demonstrates that CARS microscopy may help overcome current obstacles in relation to the assessment of NM cellular uptake and biodistribution. The applicability of the findings to other cell and NM types needs to be investigated in the future to better understand the benefits and limitations of this imaging modality. In conclusion, we believe CARS microscopy is a powerful tool that can be used to investigate the uptake of NMs by cells and tissues from in vitro and in vivo studies which has the added advantage that it requires minimal sample preparation.

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Photonic portable detection system to evaluate the oxidative potential of airborne nanoparticles and related oxidative stress in exhaled air

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Of the unintentional routes of human exposure to particulates, inhalation is one of the most significant ones. The lungs are an efficient entry portal for a variety of gaseous and aerosol-transported molecules, providing a large surface area and thin epithelial barrier, in addition to extensive vasculature. Inhalation of nanoparticles has already been documented to induce oxidative stress associated with the development of a plethora of pulmonary and cardiovascular diseases. Recent studies have shown that oxidative potential of nanoparticles are a key parameter able to induce oxidative stress in exposed cells (Burello, 2011). Among the different (bio)markers of oxidative stress processes within the lungs, hydrogen peroxide (H2O2) is a main reactive oxygen species (ROS) target but efforts to standardize its detection in the exhaled air/EBC are needed. In that context, we have developed a novel photonic system for the sensitive detection of H2O2 able to quantify both the oxidative potential of airborne nanoparticles and the H2O2 content in exhaled air. The core of the system relies on the following physical principle: multiple scattering occurring into porous material enables optical path elongation and thus absorbance enhancement (Suárez et al. PCT/IB2011/0558192011). Therefore, the functionalisation of a scattering random medium (e.g. glass fiber membrane or microbeads aggregates) with H2O2-sensitive (bio)molecules such as cytochrome c or Fe(II)/orange xylenol (FOX) gives rise to detection devices with enhanced sensitivity (40-folds) and particularly low limit-of-detection at the nano-/sub-nanoMolar range (Suárez, 2013; Suárez, 2014). With the present system the reactivity of a series of aerosolized nanoparticles can be determined (in H2O2 equivalents) leading to their classification. In another version, the aerosol is eluted into any solution of choice (e.g. different pH) and the assay reagents are added in-line between the elution chamber and the photonic sensor. Concerning the exhaled air analysis, the detection system is currently being validated in lab with the final objective of on-site sensitive measurements on exposed workers.

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Batch-to-batch reproducibility - a challenge for safety assessment and regulation

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