Non-invasive methods and biological models for nanotoxicity assessement

RISK GONE

WP5: T5.2, T5.3 & WP6: T6.1, T6.2

Nanomaterials (NMs) can interfere with dyes and reagents used in conventional toxicity assays, biasing their results. This prompts the search for interference-free high-throughput screening methods to evaluate the toxicity of NMs.

Impedance-based methods are virtually interference-free and enable rapid and multiparametric analyses. We have employed ***real-time impedance-based monitoring*** (xCELLigence system) and **impedance-based flow cytometry,** the latter established by our group, to assess the effects of NMs on cell death and proliferation. For oxidative stress evaluation, we have established ***Cyclic Voltammetry-based methods***. To investigate the effects of NMs under in vivo-like conditions, we have developed an ***impedance-based microfluidic system*** and a ***3D microvasculature model***. ***Modelling and results' interpretation*** is performed in collaboration with the Western Norway University of Applied Sciences (**HVL**) partner.

The NM dispersions were performed according to WP4 and the NANOGENOTOX protocols. The HDD, PDI, and Z-potential) were measured at the beginning and at the end of exposure.

WP5 T5.1 –Colony Forming Efficiency: Round Robin 1 (RR1) and RR2 – results presented in D5.1.

WP2, WP5 and WP6 – bibliographic analyses for review papers.

RESULTS

Task 5.2: Comparison of HTS & HCA methods for human hazard assessment

Table 1. Number of HTS/HCA publications with a GuideNano Q score ≥ 0.8: Publications are sorted according to endpoint.

Endpoint	Nr. of HTS publications		Nr. of HCA publications	
	Total	Q score ≥ 0.8	Total	Q score ≥ 0.8
Cytotoxicity	8	4	14	2
Genotoxicity	17	13	1	1
Adverse outcome pathway	10	5	10	3
Oxidative stress	0	0	11	6
Lysosomal disfunction	0	0	4	1
Inflammation	-	-	-	-

Table 2. Cytotoxicity methods grouped according to endpoint

Method (partner)	Endpoints addressed	HTS / HCA
Bioimpedance (UiB)	Proliferation, viability	HTS
Cell count (ANSES)	Proliferation, viability	HCA
Nuclear intensity	Proliferation, viability	HCA
Nuclear size	Proliferation, viability	HCA
WST-1 (KUL)	Metabolic activity	HTS
Alamar blue (NILU)	Metabolic activity	HTS

Table. 3. Cell types tested per endpoint

Method (partner) Cytotoxicity	Endpoints addressed	Cell type tested
Bioimpedance (UiB)	Proliferation, viability	A549
Cell count (ANSES)	Proliferation, viability	A549
Nuclear intensity	Proliferation, viability	A549
Nuclear size	Proliferation, viability	A549
WST-1 (KUL)	Metabolic activity	A549 & TK6
Alamar blue (NILU)	Metabolic activity	A549 & TK6

Task 6.2: HTS methods for ecotoxicity testing

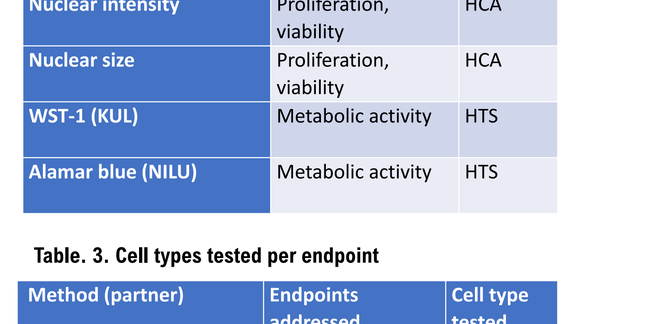

Figure 1. Impedance-based real-time monitoring (xCELLIgence, ACEA Biosyst.) of RT-GC cells: Cell proliferation and viability (Cell Index) after exposure to TiO_2 (JRC), CuO (PlasmaChem) and ZnO (Sigma).

For more results see HVL poster (E Cimpan) and D6.2.

R&D METHODS

Impedance-based flow cytometry (IFC)

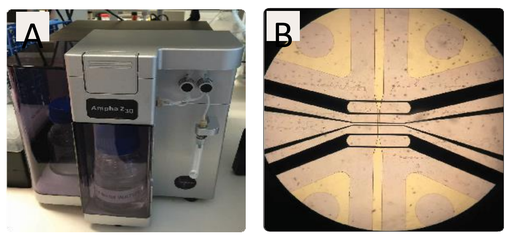

Figure 2. Impedance-based flow cytometry (IFC). A) Commercial IFC instrument (Ampha Z30, Amphasys,). B) Microfluidic chip with integrated sensing electrodes for IFC analysis.

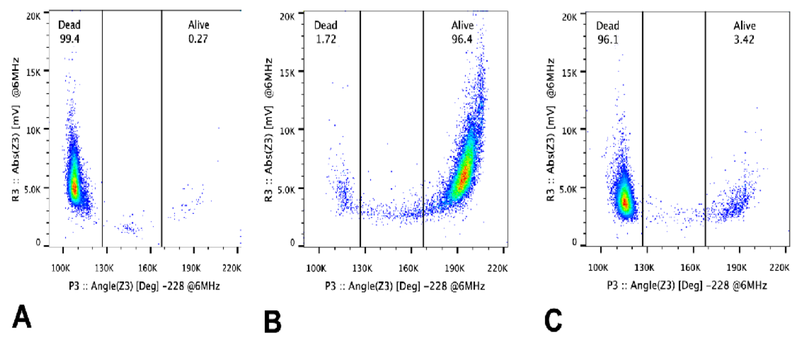

Figure 3 . Impedance flow cytometry (Ampha Z30) of U937 cells exposed to spherical Ag NMs(JRC, NM-300K, < 20 nm) with controls: (A) necrotic cells, (B) viable cells, and (C) cells exposed to Ag particles. Applied settings: Trigger level 0.02, Modulation 3, Amplifier 3, Demodifier 2, and pump speed 100 rpm.

Cyclic Voltammetry (CV)

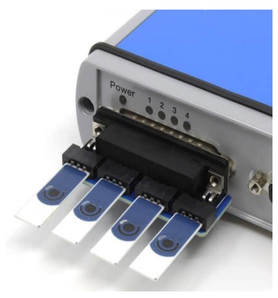

Figure 4. Cyclic voltammetry (CV) for label-free analysis of NM effects. CV voltage is swept from one value to another, and then back again, resulting in a current as a function of voltage and scan rate. It provides information about electrochemical reactions (redox) occurring at the working electrode. CV can be used to monitor oxidation and metabolism of ascorbic acid (AA).

For more information see posters of I Rios_Mondragon (UiB) and E Cimpan (HVL) and D5.2, D6.2.

Impedance-based Microfluidic system for live impedance-based and microscopy monitoring

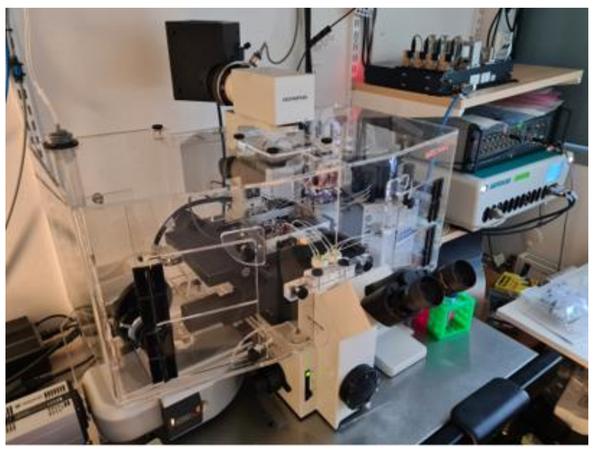

Figure 5. Microfluidic setup consisting of a microscope, DSD2 unit and camera enclosed in an environmental chamber. Inside the chamber there is a microscope-stage for the microfluidic chip, the tubing-system containing reservoirs and CO_2-tubes and the peristaltic pump

Microvasculature

Figure 6. Top view of the design of the microvasculature chip. The microfluidic chip consists of three channels, where the middle channel is for co-culturing endothelial cells with fibroblasts in a fibrin gel to induce in vitro angiogenesis, while the outer two channels are for culturing endothelial cells, thereby creating endothelial lining.

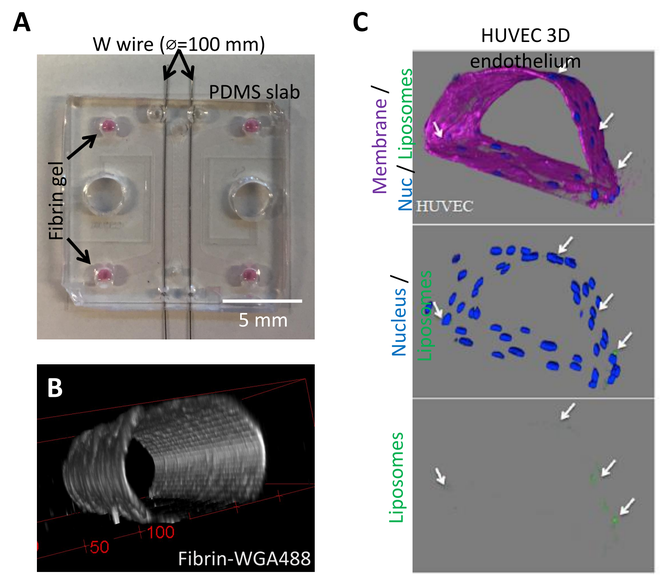

Figure 7. Microvascular model for the study of NM transport. A) Microfluidic chip made in polydimethylsiloxane (PDMS) with fibrinogen filling the side channels and W wires. B) 3D reconstruction of fluorescently-labelled Fibrin gel. Upon Fibrin gelling, the W wires are removed leaving an empty fibrin tube that supports growth of endothelial cells. C) 3D reconstruction of HUVEC microvasculature. Liposomes (Lip-SiR) were perfused in the HUVEC channel for 24 hr. The nucleus is shown in blue (DAPI), membrane in magenta (WGA-AF488) and liposomes in green. White arrows indicate Lip-SiR signal.

CONCLUSIONS

- *Bioimpedance has emerged from the interlaboratory comparison of methods as a promising HTS method for proliferation and viability screening.* It has the advantages of being label-free and thus, less prone to ENM interference, monitors cells in real-time, making it easy to identify timepoints and concentrations of interest, and less pollutant for the environment.
- *A general recommendation* is to use at least two different methods that are based on different principles for each biological endpoint tested, in order to ensure that the results are reliable.

Ivan RIOS-MONDRAGON, Ole-Bendik HOFSHAGEN, Håkon VAN TA, Marti BUSQUETS, Victor PUNTES, Zhiling GUO, Iseult LYNCH, Emil CIMPAN & Mihaela Roxana CIMPAN
University of Bergen

Final Consortium meeting
Madrid, 15-16 June 2023

This project has received funding from the European Union's Horizon 2020 research and innovation programme under agreement No814425