NANOMEDICINE GROUP AND LBCAM LAB @TTMI Publications Last 6 Months

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Utilisation of Nanoparticle Tracking Analysis

1. **Benchmark of Nanoparticle Tracking Analysis on Measuring Nanoparticle Sizing and Concentration**

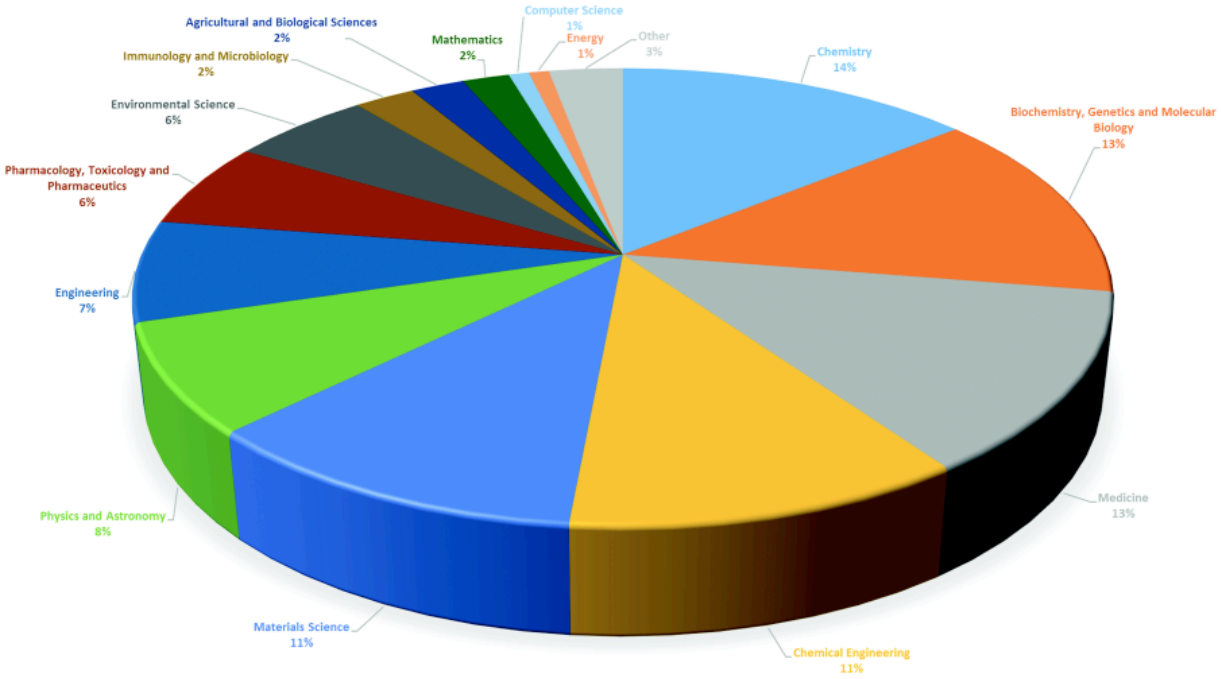
   Ciarán M. Maguire, Katherine Sillence, Matthias Roesslein, Claire Hannell, Guillaume Suarez, Jean-Jacques Sauvain, Sonja Capracotta, Servane Contal, Sebastien Cambier, Naouale El Yamani, Maria Dusinska, Agnieszka Dybowska, Antje Vennemann, Laura Cooke, Andrea Haase, Andreas Luch, Martin Wiemann, Arno Gutleb, Rafi Korenstein, Michael Riediker, Peter Wick, Patrick Hole and **Adriele Prina-Mello**
   Journal of Micro and Nano-Manufacturing, (2017) 5(4), 041002 DOI: 10.1115/1.4037124

One of the greatest challenges in the manufacturing and development of nanotechnologies is the requirement for robust, reliable, and accurate characterization data. Following engagement with Malvern Instruments Ltd, and ten pan-European research facilities, the size and concentration characterization of nanoparticles in liquid suspension was proven to be robust and reproducible for multiple sample types in monomodal, binary, or multimodal mixtures. The limits of measurement were shown to exceed the 30–600 nm range (with all system models), with percentage coefficients of variation (% CV) being calculated as sub 5% for monodisperse samples. Particle size distributions were also improved through the incorporation of the finite track length adjustment (FTLA) algorithm, which most noticeably acts to improve the resolution of multimodal sample mixtures. The addition of a software correction to account for variations between instruments also dramatically increased the accuracy and reproducibility of concentration measurements. When combined, the advances brought about during the ILC allow for the simultaneous determination of accurate and precise nanoparticle sizing and concentration data in one measurement.

This work in conjunction with previous studies [1], has positioned TCD at the forefront of particle characterisation in Ireland, Europe and worldwide. Recently we have begun work

[1] Hole, P., K. Sillence, C. Hannell, C. Maguire, M. Roesslein, G. Suarez, S. Capracotta, Z. Magdolenova, L. Horev-Azaria, A. Dybowska, L. Cooke, A. Haase, S. Contal, S. Manø, A. Vennemann, J.-J. Sauvain, K. Staunton, S. Anguissola, A. Luch, M. Dusinska, R. Korenstein, A. Gutleb, M. Wiemann, A. Prina-Mello, M. Riediker and P. Wick (2013). "Interlaboratory comparison of size measurements on nanoparticles using nanoparticle tracking analysis (NTA)." Journal of Nanoparticle Research **15**(12): 1-12.

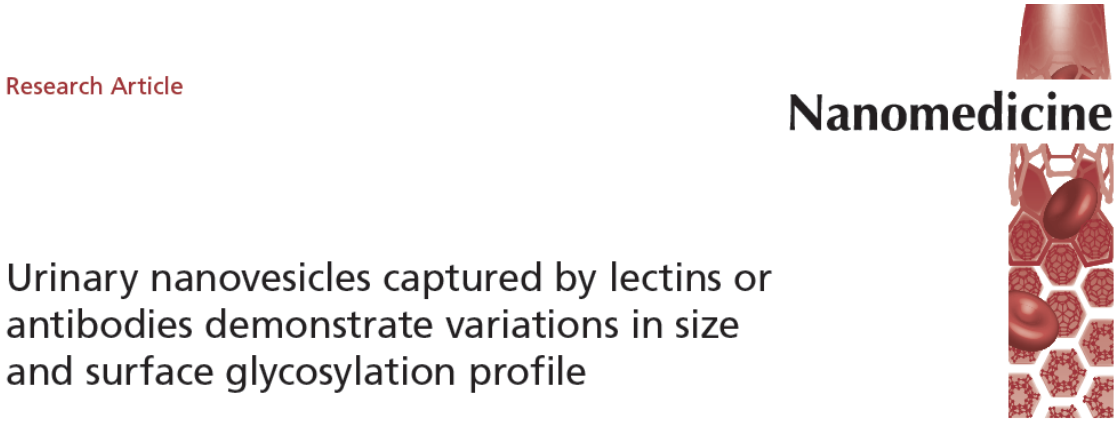
with the National Physical Laboratory (NPL) in London to further refine the measurement of nanoparticle concentrations. Similarly, our work with the US and EU Nanotechnology Characterisation Laboratories (US-NCL, EU-NCL) is allowing for the translation of advanced characterisation techniques towards industry use



Courtesy of ASME Journal of Micro and Nano-Manufacturing

CHARACTERISATION of NANOVESICLES

2. **Urinary nanovesicles captured by lectins or antibodies demonstrate variations in size and surface glycosylation profile**
   Jared Q Gerlach, **Ciaran M Maguire**, Anja Krüger, Lokesh Joshi, **Adriele Prina-Mello** & Matthew D Griffin
   Nanomedicine (Lond.), (2017) 12(11), 1217–1229 DOI 10.2217/nnm-2017-0016



Due to their physical dimensions NTA can be used in the characterisation of extracellular (EVs) and nano-vesicles. EVs have potential value in diagnostic, prognostic and therapeutic applications. Because EVs display complex carbohydrates, lectins have been proposed for the capture of EVs from a variety of biological matrices including urine. In this study, pre-concentrated urinary EVs from multiple healthy donors were pooled and exposed to lectin-conjugated or antibody(Ab)-conjugated beads. Recovered EVs were evaluated by protein estimation, transmission electron microscopy (TEM), nanoparticle tracking analysis (NTA) and lectin microarray profiling (LMP). Yields from lectin and Ab-based affinity capture were small, but the physical characteristics imply that such affinity-captured EVs may be subpopulations which vary in size and surface content. TEM confirmed similar EV diameters to those established by NTA, but total particle counts did not correlate closely with protein-based quantification. LMP demonstrated capture-dependent differences in surface glycosylation. Lectin affinity capture approaches may prove useful for enriching specific subsets of uEVs of particular clinical value.

Courtesy of Nanomedicine

Effect of Cell Culture Environment on Cell Behaviour

1. Culturing substrates influence the morphological, mechanical and biochemical features of lung adenocarcinoma cells cultured in 2D or 3D
   Adriele Prina-Mello, Namrata Jain, Baiyun Liu, Jason I Kilpatrick, **MA Tutty**, Alan P Bell, Suzanne P Jarvis, **Yuri Volkov, Dania Movia**
   Tissue and Cell, (2018), 50:15–30, doi.org/10.1016/j.tice.2017.11.003

Culturing substrates influence the morphological, mechanical and biochemical features of lung adenocarcinoma cells cultured in 2D or 3D

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ABSTRACT

Alternative models such as three-dimensional (3D) cell cultures represent a distinct milestone towards capturing the realities of cancer biology *in vitro* and reduce animal experimentation in the preclinical stage of drug discovery. Significant work remains to be done to understand how substrates used in *in vitro* alternatives influence cancer cells phenotype and drug efficacy responses, so that to accurately link such models to specific *in vivo* disease scenarios.

Our study describes how the morphological, mechanical and biochemical properties of adenocarcinoma (A549) cells change in response to a 3D environment and varying substrates. Confocal Laser Scanning (LSCM), He-Ion (HIM) and Atomic Force (AFM) microscopies, supported by ELISA and Western blotting, were used. These techniques enabled us to evaluate the shape, cytoskeletal organization, roughness, stiffness and biochemical signatures of cells grown within soft 3D matrices (PuraMatrix™ and Matrigel™), and to compare them to those of cells cultured on two-dimensional glass substrates. Cell cultures are also characterized for their biological response to docetaxel, a taxane-type drug used in Non-Small-Cell Lung Cancer (NSCLC) treatment. Our results offer an advanced biophysical insight into the properties and potential application of 3D cultures of A549 cells as *in vitro* alternatives in lung cancer research.

2. The curious case of how mimicking physiological complexity in *in vitro* models of the human respiratory system influences the inflammatory responses. A preliminary study focused on gold nanoparticles
   Movia D., Di Cristo L., Alnemari R., McCarthy J. E., Moustaoui H., Lamy de la Chapelle M., Spadavecchia J., **Volkov Y., Prina-Mello A**.
   Journal of Interdisciplinary Nanomedicine, (2017), 2, 2, 110–130.

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ORIGINAL ARTICLE

The curious case of how mimicking physiological complexity in in vitro models of the human respiratory system influences the inflammatory responses. A preliminary study focused on gold nanoparticles

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Keywords
Gold nanoparticles, inflammation, in vitro complexity, monocyte recruitment assay.

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ABSTRACT

Environmental and biomedical nanoparticles can pose potential health risks to the human respiratory system by inducing severe lung inflammation. The aim of this case study is to present a comparison of the inflammatory response in four in vitro models of the human lung epithelium, differing by composition and/or culturing substrates, when exposed to gold nanoparticles (AuNPs). Three in vitro models of lung adenocarcinoma (A549) cells and a commercially available three-dimensional (3D) culture (MucilAir™) were tested. The models were exposed to AuNPs for 3, 6, and 24 h. AuNPs internalisation was investigated by confocal, electron microscopy, and Raman spectroscopy. Enzyme-Linked Immuno-Sorbent Assay (ELISA) was used for quantifying the secretion of the inflammatory mediator Interleukin-6 (IL-6) following exposure to AuNPs. Finally, a microfluidic approach was developed in-house to investigate whether pro-inflammatory mediators present in supernatants harvested from the AuNPs-treated cell cultures could trigger monocyte activation. Our results demonstrated that AuNPs were internalised only in submerged cultures grown on glass substrates. Nevertheless, AuNPs internalisation did not trigger a significant IL-6 secretion. Significant amounts of IL-6 were secreted by AuNPs-treated mono-cultures grown on Transwell™ inserts, triggering monocyte activation in dynamic microfluidic experiments. AuNPs did not induce IL-6 secretion in co-cultures and MucilAir™

3. Nanotoxicity in Cancer Research: Technical Protocols and Considerations for the Use of 3D Tumour Spheroids
Movia D. & Prina-Mello A.
In book: Nanotoxicity, 2017, Publisher: InTech, Editors: Ferreira de Castro Gomes Andreia, Sárria Pereira Passos Marisa. ISBN 978-953-51-5392-4. In press.

4. Characterization of SH-SY5Y human neuroblastoma cell growth over glass and SU-8 substrates
   A Ajetunmobi, D McAllister, N Jain, O Brazil, A Corvin, Y Volkov, D Tropea, Prina-Mello A.
   Journal of Biomedical Materials Research Part A, (2017), 105A:2129–2138 DOI: 10.1002/jbm.a.36071

These publications reflect the changes that can occur when cells are cultured in non-traditional settings, such as in 3D or on novel substrates.

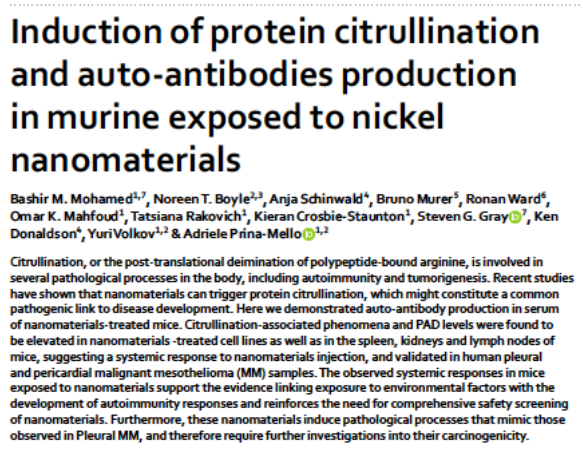
3D cell cultures represent a distinct milestone towards capturing the realities of cancer biology in vitro and reduce animal experimentation in the preclinical stage of drug discovery. The growth of cells in 3D culture can influence their shape, morphology and biochemical properties. 3D cell models that show a satisfactory correlation to clinical scenarios could in fact be used for an effective preclinical efficacy validation of novel anticancer drugs, ultimately providing a perspective platform for personalised medicine approaches. Our study can therefore find direct application in any attempt to relate the pathological condition/process of interest with the appropriate 3D system.

In the market, there are several products available for spheroids preparation; however, for many of them operators are left with the burden to optimise the working protocols to their specific needs. To support the identification of candidates with clinical potential, in a recently published book chapter we have provided protocols for the collection of quantitative data on the efficacy and safety of drug nanocarriers (nanomedicine). The protocols described provide technical solutions for the formation of scaffold-free 3D spheroids and for the characterisation of their architecture and protein marker expression. No further optimisation is needed, with the additional advantage of their full compatibility with contemporary high-throughput technologies.

As stated, the physical properties of substrates can have pro-found effects on the structure and function of cultured cells. In another study, we aimed to examine the viability, adherence, and morphological and functional variations between SH-SY5Y human neuroblastoma cells cultured on SU-8 surfaces com-pared with control surfaces composed of borosilicate glass, which are routinely used for cell culture. The SU-8 polymer has been extensively studied for its biocompatibility, but there has been little investigation into the characteristic differences between cells cultured on SU-8 when compared with glass. Results showed that SH-SY5Y cells grown on SU-8 have significantly improved viability and increased morphological and functional characteristics of neurodevelopment. The results from this study suggest that the mechanical properties of the polymer are optimal for the study of cultured cell lines, which could account for the increased viability, adherence, and morphological and functional characteristics of neurodevelopment.

Response of Cells to Nanomaterials

1. Induction of protein citrullination and auto-antibodies production in murine exposed to nickel nanomaterials
   Bashir M. Mohamed, Noreen T. Boyle, Anja Schinwald, Bruno Murer, Ronan Ward, **Omar K. Mahfoud, Tatsiana Rakovich, Kieran Crosbie-Staunton**, Steven G. Gray, Ken Donaldson, **Yuri Volkov & Adriele Prina-Mello**
   Scientific reports, (2018) 8:679 DOI:10.1038/s41598-017-19068-1



Induction of protein citrullination and auto-antibodies production in murine exposed to nickel nanomaterials

Bashir M. Mohamed[1,7], Noreen T. Boyle[2,3], Anja Schinwald[4], Bruno Murer[5], Ronan Ward[6], Omar K. Mahfoud[1], Tatsiana Rakovich[1], Kieran Crosbie-Staunton[1], Steven G. Gray[7], Ken Donaldson[4], Yuri Volkov[1,2] & Adriele Prina-Mello[1,2]

Citrullination, or the post-translational deimination of polypeptide-bound arginine, is involved in several pathological processes in the body, including autoimmunity and tumorigenesis. Recent studies have shown that nanomaterials can trigger protein citrullination, which might constitute a common pathogenic link to disease development. Here we demonstrated auto-antibody production in serum of nanomaterials-treated mice. Citrullination-associated phenomena and PAD levels were found to be elevated in nanomaterials -treated cell lines as well as in the spleen, kidneys and lymph nodes of mice, suggesting a systemic response to nanomaterials injection, and validated in human pleural and pericardial malignant mesothelioma (MM) samples. The observed systemic responses in mice exposed to nanomaterials support the evidence linking exposure to environmental factors with the development of autoimmunity responses and reinforces the need for comprehensive safety screening of nanomaterials. Furthermore, these nanomaterials induce pathological processes that mimic those observed in Pleural MM, and therefore require further investigations into their carcinogenicity.

2. Cadmium nanoparticles citrullinate cytokeratins within lung epithelial cells: cadmium as a potential cause of citrullination in chronic obstructive pulmonary disease?
   Hutchinson D., Müller J., McCarthy J.E., Gun'ko Y., Verma N., Bi X., **Di Cristo L., Kickham L., Movia D., Prina-Mello A., Volkov Y.**
   Int J Chron Obstruct Pulmon Dis., 2018, 13: 441-449; DOI: 10.2147/COPD.S152028

Objective: The objective of the study was to determine whether the cadmium-derived materials induce intracellular protein citrullination.
Methods: Human A549 lung epithelial cells were exposed to cadmium in soluble and nanoparticulate forms represented by cadmium chloride ($CdCl_2$) and cadmium oxide (CdO), respectively, and their combinations with ultrafine carbon black (ufCB) produced by high temperature combustion, imitating cigarette burning. Protein citrullination in cell lysates was analyzed by Western immunoblotting and verified by immunofluorescent confocal microscopy. Target citrullinated proteins were identified by proteomic analysis.
Results: CdO, ufCB and its combination with $CdCl_2$ and CdO after high temperature combustion induced protein citrullination in cultured human lung epithelial cells, as detected by immunoblotting with anti-citrullinated protein antibody. Cytokeratins of type II (1, 2, 5, 6A, 6B and 77) and type I (9, 10) were identified as major intracellular citrullination targets. Immunofluorescent staining confirmed the localization of citrullinated proteins both in the cytoplasm and cell nuclei.
Conclusion: Cadmium oxide nanoparticle exposure facilitated post-translational citrullination of proteins.
Keywords: cadmium, COPD, nanoparticles, cytokeratins, citrullination, autoimmunity, proteomics

3. Industrial grade 2D Molybdenum Disulphide (MoS_2): An *in vitro* exploration of the impact on cellular uptake, cytotoxicity, and inflammation

Moore C., **Movia D.**, Smith R., Hanlon D., Lebre F., Lavelle E., Byrne H., Coleman J., **Volkov Y.**, McIntyre J
2D Materials, (2017), 4, 025065.

Although exciting technological developments and nanomedical applications are emerging from the nanotechnology field, it has become important to consider any potentially detrimental impacts of these materials on human health and the environment, giving rise to the field of nanotoxicology.
Citrullination, or the post-translational deamination of polypeptide-bound arginine, is involved in several pathological processes in the body, including autoimmunity and tumorigenesis. Recent studies have shown that nanomaterials can trigger protein citrullination, which might constitute a common pathogenic link to disease development. In these publications, we demonstrated auto-antibody production in serum of nanomaterials-treated mice, as well as in human lung epithelial cells, in response to nanomaterials exposure. Citrullination-associated phenomena and PAD levels were found to be elevated in nanomaterials -treated cell lines as well as in the spleen, kidneys and lymph nodes of mice, suggesting a systemic response to nanomaterials injection, and validated in human pleural and pericardial malignant mesothelioma (MM) samples. The observed systemic responses in mice exposed to nanomaterials support the evidence linking exposure to environmental factors with the development of autoimmunity responses and reinforces the need for comprehensive safety screening of nanomaterials. Furthermore, these nanomaterials induce pathological processes that mimic those observed in Pleural MM, and therefore require further investigations into their carcinogenicity.
The recent surge in graphene research has led to advancements which are accelerating the exploration of alternative 2D materials such as molybdenum disulphide (MoS_2). A comprehensive nanotoxicology study was recently carried out, providing a better understanding to the biointeraction of MoS_2 material which is produced in the manufacturing environment in non-sterile conditions, with results confirming MoS_2 nanoflakes of three sizes at a concentration of 1 μg ml^{-1} are non-toxic in three cell lines even in the presence of LPS contamination.

Policy Making

1. **NANoREG framework for the safety assessment of nanomaterials**

Stefania Gottardo, Maria Alessandrelli, Valeria Amenta, Rambabu Atluri, Grazia Barberio, Cindy Bekker, Philippe Bergonzo, Eric Bleeker, Andy M. Booth, Teresa Borges, Patrizia Buttol, David Carlander, Stefano Castelli, Sylvie Chevillard, Simon Clavaguera, Susan Dekkers, Camilla Delpivo, Paola Di Prospero Fanghella, Maria Dusinska, Juha Einola, Elina Ekokoski, Carlos Fito, Helena Gouveia, Romain Grall, Karl Hoehener, Paula Jantunen, Gunnar Johanson, Peter Laux, Hans Christian Lehmann, Riitta Leinonen, Agnieszka Mech, Christian Micheletti, Cornelle Noorlander, Mats Olof-Mattsson, Agnes Oomen, Laia Quiros Pesudo, Maria Letizia Polci, **Adriele Prina-Mello**, Kirsten Rasmussen, Hubert Rauscher, Araceli Sanchez Jimenez, Juan Riego Sintes, Simona Scalbi, Jacques-Aurélien Sergent, Helene Stockmann-Juvala, Myrtill Simko, Adriënne Sips, Blanca Suarez, Abdelqader Sumrein, Martie van Tongeren, Socorro Vázquez-Campos, Nádia Vital, Tobias Walser, Susan Wijnhoven, Hugues Crutzen

EC JRC POLICY AND SCIENCE; EUR 28550 EN, doi 10.2760/245972

JRC SCIENCE FOR POLICY REPORT

NANoREG framework for the safety assessment of nanomaterials

The present report has been developed within the NANoREG project: "A common European approach to the regulatory testing of nanomaterials", funded by the European Union's 7th Framework Programme, under grant agreement N° 3105841. The objective of this report is to disseminate the "NANoREG framework for the safety assessment of nanomaterials" that has been developed within the project via a collective effort of several partners and supported by a NANoREG-wide consensus. JRC, as task leader, has coordinated the drafting process as well as edited and published the

document. The NANoREG framework represents the project's proposal for a common understanding in the field of environmental health and safety (EHS) assessment of nanomaterials (NMs) under the current European regulatory framework, with focus on the REACH Regulation 1907/2006. It is at the same time a contribution to the on-going debate on the need to facilitate the implementation of REACH for NMs. The framework elaborates the further development, testing and validation of three forward-looking strategies on EHS of NMs, such as Safe-by-Design, Life Cycle Assessment, and a Nanospecific approach to Prioritisation and Risk Assessment. The NANoREG partners, including JRC, believe that the proposed framework will be useful for scientific experts and stakeholders, such as regulatory authorities and industry. This report contributed to the discussion paper prepared for the ProSafe & OECD Joint Conference held in Paris from 30 November to 2 December 2016. This report is contributing to the development of the ProSafe Task Force White Paper scheduled for release in May 2017. The framework consistently uses the NANoREG harmonised terminology for EHS assessment of NMs developed by the project and illustrated in a previous JRC report released in March 20162 . This document is interlinked to other NANoREG outputs, including the questions of regulatory relevance in the field of EHS of NMs and (elements of) answers to those questions, resulting from the research work by numerous project partners (NANoREG deliverables D1.01 3 and D1.09 4). Moreover, the framework is closely connected to the comprehensive NANoREG toolbox that is available in an Excel format (project deliverable D1.125). A peer-reviewed publication describing the structure and content of the toolbox is currently under preparation.

Recently funded projects

Topic	Funding agency	Project title	Duration
Effect of Cell Culture Environment on Cell Behaviour	**Johns Hopkins** University Center for Alternatives to Animal Testing (CAAT)	Pre-clinical models for predicting the toxicity, absorption and biodistribution of inhaled nanomaterials in humans	2018-2020
Response of Cells to Nanomaterials / Policy Making	**European Commission** (EU H2020 programme; call: NMBP-12-17)	**BIORIMA - : BIOmaterial RIsk Management**	2017-2021
		REFINE	