June 2020

Project News

Press Release 3— June 2020

Developing an Integrated Risk Management Framework for Nano-BioMaterials used in medical devices and advanced therapeutic medicinal products

BIO RIMA

Latest news from BIORIMA after 30 months!

In these times, when COVID-19 is still challenging the world, we all have opportunities to step back and see things from a different perspective. And indeed, many of us are experiencing this pandemic not only as a challenge, but also as a time for change and renewal, be it on the political, socioeconomic or personal level, and in particular related to our existing safety and health care systems. This pandemic emphasizes the fundamental and urgent need to develop highly innovative intelligent, and sustainable ways to be prepared for unexpected future events and to better meet the risks that may come along with them.

The increasing convergence of cutting-edge technologies, such as nanotechnology and biotechnology, is leading to completely new advancements and breakthroughs in medical applications, including imaging, diagnostics, therapeutics, and regenerative medicine. These innovations are very much based on the development of novel nanoengineered biomaterials (NBMs), that increasingly include bio-active and bio-mimetic materials. However, there are concerns that these new advanced materials may give rise to human health and/or environmental risks. For this reason, manufacturers must establish a risk management plan for each medical device, to identify and analyze any known and foreseeable hazards, estimate and evaluate associated risks, and show how to eliminate or control these risks, when applying for a marketing authorization of a medical device or product (according to European regulation, such as REACH or MDR 2017/745).

BIORIMA is an interdisciplinary European research initiative, and the only one of this kind to develop robust and validated methodologies to assess and manage any risks of NBMs when used in medical devices (MD) and advanced medicinal therapy products (AMTP), beyond clinical tests, including safe-by-design strategies to minimize or eliminate these risks. A comprehensive testing and method validation program has been set up to improve the applicability of existing tools and approaches for human and environmental hazard and exposure assessment to NBM. The developed methodologies are integrated into a risk management framework (RMF) and a web-based Decision Support System that guides end-users to comply with existing regulations by finding the best practical solutions for testing and handling of NBMs.

This 3rd press release will update you on the most recent progress and achievements obtained within BIORIMA in this field.
We will highlight some of the latest research activities and outcomes that have been generated during the last 12 months within the project and hope they may attract your interest and serve you in your daily business and research. For a more complete view of the R&D activities that have been performed so far in BIORIMA, please visit our website (www.biorima.eu), follow us on Twitter: @biorimaproject and LinkedIn, or visit our new stakeholder forum at www.biorima.eu/forum, where you can get in direct contact with our experts, post a question or any topic you want to discuss, or just give us your feedback.

Best regards
Rudolf Reuther (Editor)
Lesley Tobin (Production)

The project has received funding from the European Union's Horizon 2020 research and innovation programme under grant agreement No 760928.

Trinity College, Dublin

When assessing the hazard and risk of NBMs, the first step is to determine their acute toxicity. As inhalation is a major exposure route for humans, the respiratory epithelium is the first tissue that inhaled NBMs may directly affect.

Acute inhalation toxicity testing is currently performed in rats and/or mice according to OECD TG403, TG436, and TG433 guidelines. However, as these tests are biased by differences in the respiratory tract architecture and function across species, it is difficult to draw conclusions on the true hazard of inhaled compounds in humans.

BIORIMA researchers from the Trinity College Dublin (TCD) have come up with a completely new perspective to support the development and validation of alternative, human-relevant, *in vitro* models as regulatory accepted alternative for inhalation animal testing (https://www.frontiersin.org/articles/10.3389/fbioe.2020.00549/full). This includes a checklist of key parameters that should be reported in future scientific publications related to the reproducibility and transparency of results. In addition, a new 3D Multilayered Cell Culture (MCCs) model has been created and successfully tested based on human NSCLC (A549) cells and grown at the Air-Liquid Interface (ALI) as the first *in vitro* tool for screening the efficacy of inhaled anti-cancer drugs https://bmccancer.biomedcentral.com/articles/10.1186/s12885-019-6038-x).

These studies will open up new research avenues for the development of alternatives to animal-based inhalation studies.

Contact: Dania Movia

University of Paris

The group of Prof. Armelle Baeza from University of Paris has developed a new reliable *in vitro* model that allows the screening of multiple NBMs and doses and repeated exposure to NBM, to study long-term exposure and effects (after 28 days). The cell line is cultured on porous inserts in two-compartment chambers (Transwell®) enabling an air-liquid interface to mimic the in vivo situation in the human airway epithelium.

Proteomic analysis of the apical secretomes has shown that the model shares 100% of the extracellular proteins of primary cultures of human bronchial epithelial cells. It also allows the formation of a polarized and tight epithelium that maintains this functional barrier for over a month.

The cell culture model has been successfully tested and used to evaluate long-term effects of NBM, including effects on cell viability, barrier integrity, pro-inflammatory responses, mucin production, and the ability of NBMs to cross the epithelial barrier.

It is one of the first validated *in vitro* models that ultimately provides a complete picture of the hazard of NBM in the human airway epithelium.

Contact: Sonja Boland

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Developing an Integrated Risk Management Framework for Nano-BioMaterials used in medical devices and advanced therapeutic medicinal products

University of Torino and RCSI

Much of the research in BIORIMA is devoted to identifying and using the physicochemical properties of NBMs that can modulate their behavior (such as aggregation) in the human body. Results on carbon and silica nanoparticles performed by the University of Torino showed that large rather than small carbon nanoparticles, or silica nanoparticles, show a strong tendency to aggregate both in the plasma and blood not because of platelets activation, but as a consequence of the formation of the protein corona. In addition, also the composition of the protein corona differed depending on the chemical nature of the nanoparticles. Although coagulation proteins were abundant in the corona of both silica and carbon nanoparticles, surface characteristics seem to play a minor role. The results achieved suggest that vessel occlusion and formation of thrombi *in vivo* may occur through an independent mode of action (MoA) that is strongly influenced by the physicochemical characteristics of the used NBM.

Contact: Ivana Fenoglio

Karolinska Institutet

Although Ag nanoparticles are widely used as antibacterial agents, little is known on the response of the human immune system. Prof. Bengt Fadeel and his group from KI used cell lines consisting of monocyte/macrophage and lung epithelial cells in their experiments to find an answer to this question. It was shown that Ag nanoparticles reduce the secretion of pro-inflammatory cytokines in response to LPS, likely as a result of the release of Ag ions leading to an interference with TLR signaling. Further *in vivo* studies are needed to validate the observed effects.

Contact: Bengt Fadeel

CEA

Studies on the interrelationship between toxicity and the physicochemical transformation of Ag nanoparticles (NP) in the human body by using A549 lung cells indicated the importance of dose rates. Cells exposed to high dose rates of Ag-NPs lead to severe outcomes, especially on DNA integrity. Conversely, repeated exposure to low dose rates caused a profound alteration of the cell metabolism and a complete blockage of the cell cycle progression. X-ray absorption analysis of Ag speciation confirmed that Ag-NPs progressively dissolve intracellularly and that Ag ions recombine with thiolated proteins. The results confirm that surface coating of Ag nanoparticles is a key determinant for their dissolution rate and final toxicity.

L. Bobyk, A. Tarantini, D. Beal, G. Veronesi, I. Kieffer, S. Motellier, E. Valsami-Jones, I. Lynch, P.-H. Jouneau, K. Pernet-Gallay, C. Aude-Garcia, S. Sauvaigo, T. Douki, T. Rabilloud, M. Carriere. Toxicity and chemical transformation of silver nanoparticles in A549 lung cells: genotoxic impact depends on the dose rate. *Submitted*

Contact: Marie Carriere

Electrospinning

An interesting spin-off of the development and use of proper safety-by-design measures in medical applications has been shown by the Electrospinning Company. It was shown that applying safer-by-design measures to Ag nanoparticles led to improvements of existing processes and products, and at the same time lowered the environmental, occupational, and patient safety risks caused by Ag nanoparticle bearing materials. In addition, Ag containing electrospun materials proved even to serve as effective anti-viral filter materials for face masks. BIORIMA partners are now exploring opportunities to fund the development of this and other promising applications to counter COVID-19 and other health care risks.

Contact: Sanju Malla

The project has received funding from the European Union's Horizon 2020 research and innovation programme under grant agreement No 760928.

University of Torino

That size and shape of nanoparticles are crucial for their final toxicity was demonstrated in another study by the team of Prof. Enrico Bergamaschi from the University of Torino in testing different TiO2 nanofibers (NF). Fiber shortening proved to be an effective way to mitigate adverse effects on *in vitro* cell viability and epithelial barrier integrity as well as on *in vivo* inflammation. Obtained results suggest that shortening NF may be part of as an effective safety-by-design strategy to mitigate possible toxic TiO2 NF effects.

Contact: Enrico Begamaschi

ISTEC

Researchers from ISTEC, Italy, compared the material properties of two differently synthesized NBM to demonstrate how safe-by-design measures help to reduce any associated risks. They tested a fiber-based Ag-PLLA (poly-L-lactide) that is used for wound dressing and contains pure Ag NPs (AgSigma), and a Ag NP (Ag-HEC) coated with a benign polymer (hydroxyethyl cellulose) and also embedded into the PLLA-fibers, as an alternative NBM for wound dressing.

The colloidal behavior (hydrodynamic diameter and Z-potential) and ion release (ICP-OES) of coated and uncoated Ag NPs dispersed in water and in a physiologically relevant medium (synthetic sweat) was measured and the antibacterial effects and ion release of the Ag-PLLA electrospun fibers from both types of embedded Ag nanoparticles assessed.

Tests are still ongoing by using an *ex vivo* and *in vivo* pig skin model to assess potential toxicological dermal effects.

Contact: Magda Blosi

Joanneum Research Group

The JOANNEUM RESEARCH group used Open Flow Microperfusion (OFM) to study the dermal uptake of NBMs into the human body. OFM probes were inserted into the dermal tissue and the dermal interstitial fluid was continuously sampled allowing a time-resolved monitoring of the dermal uptake of nanoparticles. By combining data from the OFM with biopsy homogenate data, they could not only assess the intracellular uptake of NBMs, but also their concentration in the interstitial fluid and systemic uptake into the blood circulation. Studies on the effect of particle coatings on unspecific immune response in the skin is still ongoing with first results expected in summer 2020.

Contact: Simon Schwingenschuh

CEA

To demonstrate and improve the performance and robustness of the developed methodologies under real conditions, measurement campaigns have been started. As an example, project partners CEA, ITENE and IUTA have conducted air measurements to assess the release of NBM inside a dental prosthesis laboratory (see figure).

Contact: Sebastien Artous

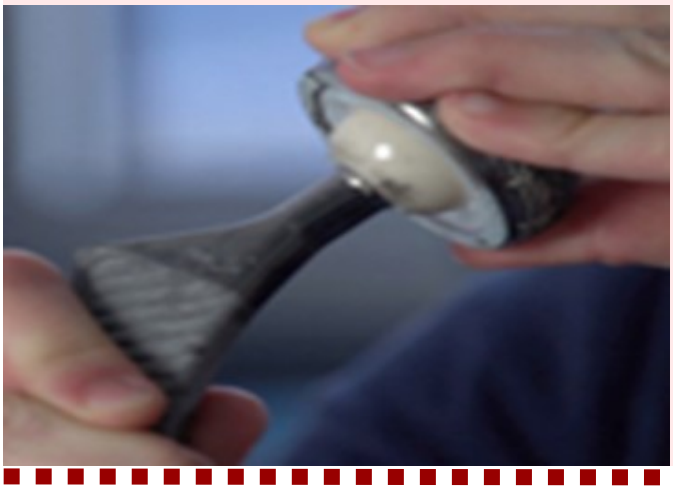
The project has received funding from the European Union's Horizon 2020 research and innovation programme under grant agreement No 760928.

Leeds University

Researchers from Leeds University took a step change in using safe-by-design to fabricate nano-enabled replacement hip joints. These LifeLongJoints (LLJ) reduce wear of the joints and toxicity from wear particles and toxic ions, and last a whole lifetime. This was achieved by a careful selection of metals, choice of metal ball on ceramic cup technology, modelling to reduce electrolytic stress corrosion generating toxic metal ions and finally by plasm coating of the nanostructured SiN of the metal shaft and ceramic cup. Wear particles from SiN were characterized and their toxicity (ROS, inflammatory, genotoxicities) tested. They proved to be biodegradable.

Two CEN Workshop Agreements (CWAs) have been obtained describing a new standard for isolation and characterisation of wear particles under clinically relevant testing conditions (CWA 17553-1) and for a complete set of *in vitro* biological methods to assess toxic effects (CWA 17253-2). Standards were used to evaluate the LLJ SiN coated replacement hip joints against about 10,000 joints available on the market.

They confirmed that LLJ joints produce far less particles and less toxicity than all other replacement hip joints currently available.



For his outstanding research in ceramic implant applications in arthroplasty, Dr. Saurabh Lal from the industrial partner “Zimmer Biomet”, won the “Heinz-Mittelmeier Research €5000 Prize” awarded by the German Society for Orthopaedic and Trauma Surgery in 2018.

Contact: Terry Wilkins

University of Torino

Research at the University of Torino provided new experimental evidence on the ability of newly synthesized carbon nanoparticles (CNP) to act as promising agent for the NIR-photo-therapy of tumors. The produced monodispersed and almost perfectly spherical CNP showed a high colloidal stability, which is required for the functionalization of a wide range of drug delivery applications.

Their uptake and cytotoxicity in cells and ability to interfere with the cellular redox homeostasis on macrophages (RAW 264.7) and with alveolar epithelial tumor cells (A549) was tested.

As a result, CNP can scavenge hydroxyl radicals (EPR spectroscopy) and so display an antioxidant activity in cells.

When irradiated with NIR (laser beam wavelength 945 nm), CNP were also able to generate heat and singlet oxygen ($^1O_2$) thus promoting cell death of tumor cells and acting as a promising photothermal and photodynamic agent for cancer treatment.

Contact: Ida Kokalari

The project has received funding from the European Union’s Horizon 2020 research and innovation programme under grant agreement No 760928.

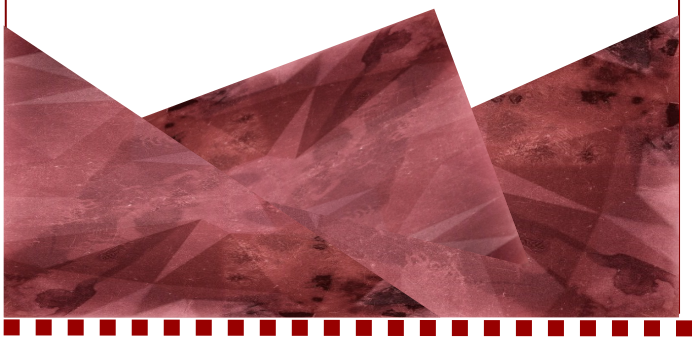
University of Venice and Green Decision

One of the main outcomes of BIORIMA will be the development and practical implementation of a risk management framework (RMF) for NBMs to help end-users to address potential risks associated with the use of these materials in medical devices (MDs) and advanced therapy medicinal products (ATMPs).

This RMF includes Integrated Strategies for Testing and Assessment (IATA) for occupational and environmental risk assessment and is implemented by a web- based decision support system (DSS). The DSS is designed to guide its users in the selection of adequate testing protocols and modeling tools for cost efficient safety assessment according to regulatory demands and stakeholder needs. In addition, the system can help to identify suitable safe by design strategies and/or risk management measures.

A first prototype of the DSS has been established by GreenDecision and is currently further developed and tested in particular by running the program with data available from industrial case studies that represent real and relevant supply chain and exposure scenarios for NBMs used in MDs (implants or protheses or for tissue regeneration) or ATMPs (for drug delivery or in vivo imaging/ biosensing). “

Contact: Danail Hristozov



RCSI

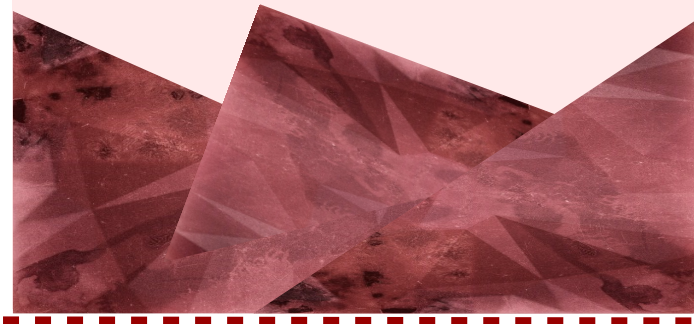
Superparamagnetic nanoparticles (NPs) are widely used in research because of their promising biomedical applications in drug delivery, hyperthermia, and MRI contrast agents. As workers or users could be indirectly exposed to these particles with unpredicted outcomes, human exposure is a matter of concern.

Regardless of the exposure route, the interaction of NPs with a biological matrix leads to the formation of a biomolecule corona that affects their biological response including accumulation, toxicity, and clearance.

Evaluating the protein corona composition and the nanomaterial properties in biological fluids is used to explore the hazard potential of NPs. Common isolation protocols such as centrifugation or 2D step electrophoresis separation are suitable to isolate magnetic NPs from biological solutions (e.g., human plasma/ serum). But these protocols are not applicable for more complex solutions such as mucus or saliva.

Dr Mahmoud Soliman from the group of Dr Monopoli at the RCSI Chemistry department, has developed a low cost, fast and effective protocol for isolating the NP-protein corona complex based on the NP magnetic properties without affecting the corona composition, which is fundamental when assessing NP toxicity.

Contact Marco Monopoli | Mahmoud Soliman



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Karolinska Institutet

In how much material properties can be used to influence the impact NBM on human health was shown in a screening study on the cytotoxicity of a set of multi-walled carbon nanotubes. Three benchmark MWCNTs from the JRC nanomaterial repository (NM-400, NM-401 and NM-402) with different morphologies have been tested in human cell lines that differentiated into macrophage- and neutrophil-like cells. NM-401, but not NM-400 or NM-402, induced caspase-dependent apoptotic cell death in macrophage-differentiated THP-1 cells. And all 3 MWCNTs produced non-apoptotic cell death in neutrophil-differentiated HL-60 cells with signs of involvement of autophagy. The results suggest that toxicological profiling may not necessarily show the same behavior of MWCNTs obtained with two different cell models. This emphasizes again the importance of using relevant cell models to understand how the potential immunotoxicity of nanomaterials interacts with different vulnerabilities of the cell models used.

Contact: Bengt Fadeel

University of Aveiro

BIORIMA is looking into environmental risks that NBMs may have. We further investigate how to adapt existing OECD guidelines for testing environmental hazard to NBM. As NBMs differ from conventional chemicals, they pose specific testing challenges. Based on experience gained in BIORIMA, changes and updates have been identified, including recommendations on how to implement it in OECD guidelines for environmental toxicity testing of NBM. We have now published this knowledge in an esteemed peer reviewed journal. Please find the all details in our golden open-access publication:

"Amorim, M.J.B., Fernandez-Cruz, M.L., Hund-Rinke, K. and Scott-Fordsmand, J.J. (in press). Environmental hazard testing of Nanobiomaterials . Environmental Sciences Europe." https://doi.org/10.1186/s12302-020-00369-8

Contact: Monica Amorim

Heriot Watt University

Due to the unique characteristics of nanobiomaterials (NBM), there has been an increase in their use in innovative medical devices (MD). These NBM-MD hold great potential as useful therapeutic and diagnostic tools.

HWU has presented at conferences and led on the production of a publication that acts as guidance to NBM-MD developers (academic and industrial) for pre-clinical tailored safety assessment. HWU has promoted the application of Integrated Approaches to Testing and Assessment (IATA) as a critical tool in developing these strategies. IATA utilise current data and a streamlined testing strategy to improve understanding of the most relevant safety risks associated with the NBM and the route of exposure. IATA aim to guide the development of pre-clinical testing strategies, allowing NBM developers to work towards current guidelines and regulations. This approach reduces the pre-clinical testing required as well as reducing the current reliance on animal models. An IATA for NBM-MDs with direct blood contact was proposed.

To further develop this IATA and others in preparation, HWU organised a successful Workshop together with GRACIOUS in Salzburg, Austria.

Contact: Vicki Stone | Teresa Fernandes

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NANOSAFE 2020

The first nanoSAFE Digital Conference
16 - 20 November 2020, online
The 7th International Conference on Health and Safety issues related to Nanomaterials for a socially responsible approach.
Register now—**Save the date!**

NanoTox 2021

10th International Conference on Nanotoxicology
20 - 22 April 2021, Edinburgh, Scotland
NanoTox2021 will be hosted by BIORIMA, GRACIOUS and PATROLS
Register now—**Save the date!**

Nanosafety Training School

From Basic Science to Risk Governance

The Interprofessional Education Training School & Young Scientist Forum that was due to take place in March this year in Venice, Italy, was unfortunately postponed due to the COVID-19 pandemic. Plans are now underway to host it in 2021, organised within the EU-funded Horizon 2020 projects BIORIMA, GRACIOUS, NanoInformaTIX, PATROLS, Gov4Nano, NanoRIGO, and RiskGone.

Keep an eye on the Biorima project website for updates.

Notes to Editors

The BIORIMA project has 43 partners from 14 different countries and is planned for four years (2017-2021). The project has received funding of almost 8 million EUR from the European Union's Horizon 2020 research and innovation programme under grant agreement No 760928.

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