Workshop

Nanotoxicology in the context of the safety assessment of nanomaterials

•

A Nanotoxicologia no contexto da avaliação da segurança de nanomateriais

2019- 11- 25

Instituto Nacional de Saúde Dr. Ricardo Jorge

Lisboa

INGESTnano
Cellular and Molecular Mechanisms of Toxicity of Ingested Nanomaterials
PTDC/SAU-PUB/29481/2017
https://www.facebook.com/INGESTnano

ToxApp4NanoCELFI
A PREDICTIVE TOXICOLOGY APPROACH TO CHARACTERIZE POTENTIAL RESPIRATORY EFFECTS OF FUNCTIONALIZED NANOCELLULOSE FIBRES IN A CO-CULTURE SYSTEM
(PTDC/SAU-PUB/32587/2017)

FCT Fundação para a Ciência e a Tecnologia

Nota Introdutória

Tem vindo a aumentar a preocupação com os potenciais riscos para a saúde humana e para o ambiente decorrentes da exposição a nanomateriais em contexto ocupacional, ambiental ou através de produtos de consumo, representando um desafio para as autoridades de saúde e ambiente, à escala mundial. Em particular, o impacto dos nanomateriais sobre o genoma humano (efeitos genotóxicos e epigenéticos) pode implicar riscos acrescidos de doenças crónicas, incluindo doenças oncológicas que se poderão manifestar apenas a longo prazo e cujo impacto ainda se desconhece. Nesta perspetiva, mostra-se necessário que investigadores, trabalhadores, profissionais da Saúde e cidadãos em geral, tomem consciência dos potenciais riscos para a saúde decorrentes da exposição a nanomateriais, bem como formas de os prevenir ou mitigar, no sentido de assegurar a Saúde Pública.

Neste *workshop*, pretende-se estimular sinergias entre projetos nacionais sobre a segurança de nanomateriais e as nanotecnologias. Os aspetos a abordar incluem a síntese e aplicação de nanomateriais, a sua potencial toxicidade, particularmente, ao nível do genoma humano, a avaliação de risco dos nanomateriais e sua aplicação em termos de avaliação e de gestão do risco. Como destinatários, os investigadores das áreas da síntese e aplicação de nanomateriais, da nanotoxicologia, das áreas da saúde e ambiente, bem como os profissionais dos setores industrial e regulamentar e cidadãos com interesse em nanotecnologias, nanotoxicologia, saúde ambiental e ocupacional. A iniciativa enquadra-se nos projetos ToxApp4NanoCELFI e INGESTnano, financiados pela Fundação para a Ciência e Tecnologia.

Data: 25 de novembro de 2019
Duração: 7 horas
Local: nas instalações do Instituto Ricardo Jorge, em Lisboa
Coordenação: Maria João Silva | Henriqueta Louro

Nanotoxicology in the context of the safety assessment of nanomaterials

INSA auditorium, Monday, 25th November 2019

Session 1	**Synthesis, application and characterization of nanomaterials. Nanotechnologies.** **Chair: Célia Ventura, Maria João Silva (INSA)**
9:00	**Challenges in Nanomaterials Characterization.** José Catita Faculty of Health Sciences • Fernando Pessoa University and Paralab SAParalab • Porto
9:30	**Nanotoxicological evaluation of biopolymeric nanocarriers: the first steps.** Ana Bettencourt, Lídia Gonçalves. Research Institute for Medicines (iMed.ULisboa) • Faculdade de Farmácia • Universidade de Lisboa
10:00	**Lipid Nanoparticles for drug deliver: preparation and validation using in vivo *Caenorhabditis elegans* model.** Luís Fonseca Instituto Superior Técnico • Universidade de Lisboa
10:30	***Coffee break***
11:00	**Topical delivery of nanostructured lipid carriers loaded with lipophilic active compounds on a 3D reconstructed human epidermis model.** Fátima Pinto Department of Human Genetics • National Institute of Health Dr. Ricardo Jorge • Lisboa
11:30	**Nanocelluloses: production, characterization and market.** Paulo Ferreira Faculty of Sciences and Technology • Department of Chemical Engineering • Coimbra
Session 2	**Nanotoxicology: concepts and methodologies for toxicity evaluation of the nanomaterials.** **Chair: Peter Jordan, Henriqueta Louro (INSA)**
12:00	**A predictive toxicology approach to characterize potential respiratory effects of functionalized nanocellulose fibres.** Maria João Silva Department of Human Genetics • National Institute of Health Dr. Ricardo Jorge • Lisboa
12:30	**New "omics" approaches as a tool to explore mechanistic nanotoxicology.** Célia Ventura Department of Human Genetics • National Institute of Health Dr. Ricardo Jorge • Lisboa
13:00	***Lunch break (not included)***
Session 2 (cont.)	**Nanotoxicology: concepts and methodologies for toxicity evaluation of the nanomaterials. (cont.) Chair: Peter Jordan, Henriqueta Louro (INSA)**
14:00	**In vitro assessment of nanomaterials hazard – how the experimental approach can make a difference.** Sónia Fraga Department of Environmental Health • National Institute of Health Dr. Ricardo Jorge • Porto
14:30	**Cellular and molecular mechanisms of toxicity of ingested nanomaterials.** Henriqueta Louro Department of Human Genetics • National Institute of Health Dr. Ricardo Jorge • Lisboa
15:00	**Nanomaterials in foods and the standardized static in vitro digestion method: contributing to the study of the potential toxic effects.** Carla Martins Department of Food and Nutrition • National Institute of Health Dr. Ricardo Jorge • Lisboa
15:30	**Using confocal microscopy for monitoring the subcellular impact of nanomaterials.** Paulo Matos Department of Human Genetics • National Institute of Health Dr. Ricardo Jorge • Lisboa
16:00	***Coffee break***
Session 3	**Risk assessment, standardization and regulation of nanomaterials.** **Chair: João Lavinha (INSA)**
16:30	**Adverse Outcome Pathways (AOPs) development, a tool for predictive nanotoxicology.** Dora Rolo Department of Human Genetics • National Institute of Health Dr. Ricardo Jorge •Lisboa
17:00	**Nanomaterials, a new challenge in the workplace.** Ana Rita Alberto Instituto de Soldadura e Qualidade • Oeiras.
17:30	**Final remarks and Closing**

INGESTnano
Cellular and Molecular Mechanisms of Toxicity of Ingested Nanomaterials
PTDC/SAU-PUB/29481/2017
https://www.facebook.com/INGESTnano

ToxApp4NanoCELFI
A PREDICTIVE TOXICOLOGY APPROACH TO CHARACTERIZE POTENTIAL RESPIRATORY EFFECTS OF FUNCTIONALIZED NANOCELLULOSE FIBRES IN A CO-CULTURE SYSTEM
(PTDC/SAU-PUB/32587/2017)

FCT Fundação para a Ciência e a Tecnologia

Abstracts – Session 1

Session 1 Synthesis, application and characterization of nanomaterials. Nanotechnologies.

Challenges in Nanomaterials Characterization

José Catita

Fac. De Ciências da Saúde-UFP; Paralab SA

In recent years, nanotechnology has been a hot topic in the scientific community due to the specific properties in the nanoscale and has become an enabling technology for numerous applications. Produced science on new nanomaterials and its characteristics and applications has been identified as a key enabling technology and keeps stimulating industrial growth, innovation and development, in the most diverse fields such as medicine, food, cosmetics, electronics, automotive, energy, construction, and other areas.

At the same time, uncertainties about its safety for human health and the environment are still hampering a more widespread exploration of its potentials and several authorities and official organisms are therefore defining actions for the implementation of a safe, integrated and responsible approach for nanoscience and nanotechnologies. As an important step in that direction, definitions of nanomaterials are being proposed and implemented for regulatory and policy purposes in order to ensure harmonized terminology and definitions across different pieces of legislation.

On the other hand, it has also been realized that the reliable detection, characterization and quantification of nanomaterials is still one of the greatest challenges particularly in complex matrices, such as products, food and the environment.

In this presentation, the most relevant techniques and protocols for the characterization of nanomaterials will be presented in regards to physical and chemical properties such as, sphere equivalent size (volume, diameter), size distribution by volume and/or number, average size (percentiles), shape, physical state (crystalline phase, amorphous, etc), surface charge, zeta potential, molecular weight, among others.

Short CV

-PharmD with a PhD in Analytical Chemistry both from the University of Oporto.
-MBA from the Catholic University Business School.
-Professor at the Faculty of Health Sciences – UFP, teaching in the fields of Biophysics, Physical-Chemistry and Advanced Analytical Techniques.
-Author or co-author of several peer reviewed publications, oral presentations and invited lectures.
-Senior Manager and Technical Director at PARALAB (Portugal, and Spain).
-Technical consultant for the development and validation of analytical methods in highly regulated industries, namely pharmaceutical industry.
-Independent Expert Evaluator at the European Commission for the evaluation of several projects related with nanomaterials characterization.
ORCID ID: https://orcid.org/0000-0001-7270-5702
Scopus Author ID: 6507275921
Loop profile: 388624
Ciência ID: B41E-3287-E706

Nanotoxicological evaluation of biopolymeric nanocarriers: the first steps

Ana Bettencourt and Lídia Gonçalves[1]

[1]Research Institute for Medicines (iMed.ULisboa), Faculdade de Farmácia, Universidade de Lisboa, Avenida Prof. Gama Pinto, 1649-003, Lisboa Portugal.

A strategy to improve the effectiveness of clinically available drugs is their encapsulation into micro- or nanoparticulate polymeric systems aiming to protect and target the drugs directly to the desired site of action for an extended period of time while reducing the systemic side effects of the drugs. However, concern related to the potential toxicity of these systems poses some questions and understanding whether the nano-scale carriers themselves may exert adverse effects is of great importance. An overview on this topic will be presented using acrylic carriers as a model system. The studies conducted by our team related to the physico-chemical characterization namely size distribution, surface charge and hydrophobicity of the systems in water as well as in relevant physiological media will be described. Also, the impact of the physico-chemical properties on the nano-biointeraction will be highlighted including the nanocarriers capacity to cross biological barriers and be uptaken by the cells. Finally, the current state of the investigation related to the use of these nanocarriers for the local delivery of antibiotics aiming the management of biomaterial associated infections will be presented.

Short CV

Ana Bettencourt (www.ff.ul.pt/~asimao) is an Assistant Professor with Aggregation at the Faculdade de Farmácia (ULisboa). She has worked in the area of nanotechnology, biomaterials and medical devices for the past 20 years and her current activities range from pharmaceutical technology, materials science to fundamental studies of cellular interactions with biomaterials including those at the nanoscale. ORCID ID 0000-0002-8498-5892.

Lídia Gonçalves is a Principal Investigator at FFUL and a team member of the NanoBB group from iMed.ULisboa. Her works focused on the development of new 3D systems (micro/nano structures) for drug delivery, including drug stabilization and targeting. She has also expertise in *in vitro* high-throughput biological assays, biotechnological process and animal experimentation. ORCID ID 0000-0002-6799-2740.

INGESTnano
Cellular and Molecular Mechanisms of Toxicity of Ingested Nanomaterials
PTDC/SAU-PUB/29481/2017
https://www.facebook.com/INGESTnano

ToxApp4NanoCELFI
A PREDICTIVE TOXICOLOGY APPROACH TO CHARACTERIZE POTENTIAL RESPIRATORY EFFECTS OF FUNCTIONALIZED NANOCELLULOSE FIBRES IN A CO-CULTURE SYSTEM
(PTDC/SAU-PUB/32587/2017)

Lipid Nanoparticles for drug deliver: preparation and validation using *in vivo Caenorhabditis elegans* model

Vera R. Esgueira, Fátima Pinto, Dragana de Barros, Jorge H. Leitão, Luis P. Fonseca

Institute for Bioengineering and Biosciences, Instituto Superior Técnico, University of Lisbon, Av. Rovisco Pais, 1049-001 Lisbon, Portugal

Today, the encapsulation of active biomolecules in liquid and solid nano- & micro-particles is a great challenge for applications on industrial biotechnology, and development of delivery carriers for cosmetic, nutraceutical and pharmaceutical applications.

The liquid heterophase systems have shown to be suitable methods for producing those materials matching the requirements of functionality and encapsulation of active and sensible biomolecules. To achieve these goals NanoLipCar Technology is the most recent development on emulsion technologies in the Institute for Bioengineering and Biosciences at IST that continues to be an excellent method and procedure for high-value applications in those industrial fields.

The production of nanostructured lipid carriers (NLC) with adequate formulation for the encapsulation of active compounds through optimization of the composition through different oils, fatty acids and surfactants was assayed using NanoLipCar Technology.

The encapsulation of vitamins and derivatives for cosmetic application and antibiotics, ciprofloxacin and tobramycin, which are currently used in Cystic Fibrosis therapy, are illustrated in this work.

The formulation chosen for the encapsulation of antibiotics were composed of stearic acid, sunflower oil, span 80 and milli-Q water characterized by an average size of 255.9±40.8nm, PdI of 0.342±0.06 and zeta potential of -56.9±3.72mV. Particles with ciprofloxacin and tobramycin had a similar average size compared to empty particles. Particles with ciprofloxacin had an encapsulation efficiency of 68.16±4.9% and a burst release where all the drug was released in first 7hours. Empty particles and particles with ciprofloxacin were stable up two months in opposition to particles with tobramycin.

The nanotoxicity and validation of encapsulation of antibiotics for Cystic Fibrosis therapy was assessed using *Caenorhabditis elegans* as an animal model of infection and *Burkholderia contaminans* IST408 and *Burkholderia cepacia* k56-2 as model of bacterial pathogens.

Short CV

Luís P. Fonseca is Associate Professor at the Department of Bioengineering of Instituto Superior Técnico (I.S.T.), Lisboa at University of Lisbon (UL).
Current research of Luis P. Fonseca focus on the use of oil-in-water emulsions in particular nanoemulsions on the design and development of encapsulation method based on hydrogels and lipid nanoparticles of a range of high-value products from flavors and fragrances, emollients, specialty and fine chemicals, and antibiotics for cosmetic, nutraceutical and pharmaceutical applications that led to the development of actual NanoLipCar Technology at I.S.T.
Email: luis.fonseca@tecnico.ulisboa.pt
Webpage: http://ibb.tecnico.ulisboa.pt/LF.html
http://orcid.org/0000-0001-8429-6977

INGESTnano
Cellular and Molecular Mechanisms of Toxicity of Ingested Nanomaterials
PTDC/SAU-PUB/29481/2017
https://www.facebook.com/INGESTnano

ToxApp4NanoCELFI
A PREDICTIVE TOXICOLOGY APPROACH TO CHARACTERIZE POTENTIAL RESPIRATORY EFFECTS OF FUNCTIONALIZED NANOCELLULOSE FIBRES IN A CO-CULTURE SYSTEM
(PTDC/SAU-PUB/32587/2017)

Topical delivery of nanostructured lipid carriers loaded with lipophilic active compounds on a 3D reconstructed human epidermis model.

Fátima Pinto[1], Luis Fonseca P[1], Sofia Souza[3], Abel Oliva[3], Dragana P C de Barros[2]

[1]National Institute of Health Dr. Ricardo Jorge (INSA), Lisbon, Portugal
[2]Institute for Bioengineering and Biosciences (iBB), Bioengineering Department/Instituto Superior Técnico (IST), Universidade de Lisboa, Av. Rovisco Pais 1, 1049-001 Lisboa, Portugal
[3]Instituto de Tecnologia Química e Biológica António Xavier (ITQB), Universidade Nova de Lisboa, Av. da Republica, 2780-157 Oeiras, Portugal

Lipid nanocarriers refer to a wide group of drug delivery systems that are well-known as effective carriers for lipophilic and hydrophilic active compounds and that can be easily integrated into dermal formulations. Nanostructured lipid carriers (NLCs) belong to the wide group of lipid nanocarriers, representing an alternative – *e.g.* to liposomes, emulsions and polymeric nanoparticles. Usually, NLCs present a spherical shape with mean diameters ranging between 50 and 500 nm and are composed by an unstructured solid lipid matrix consisting on a mixture of liquid and solid lipids, stabilized by a surfactant or a mixture of surfactants dispersed in an aqueous phase.

New optimized NLCs formulations loaded with retinyl palmitate (RP) and α-tocopherol (TOC) were evaluated regarding their topical distribution and efficacy on a 3D model of *in vitro* reconstructed human epidermis (RHE).

NLCs were produced using sunflower oil and myristic acid as liquid and solid lipids, respectively and RP and TOC as lipophilic model compounds. Also, the fluorescent dye DiO was incorporated along with TOC, resulting in a TOC-DiO-NLCs formulation to enable a qualitative characterization on *in vitro* absorption studies. Physicochemical properties of empty NLCs, RP-NLCs and TOC-DiO-NLCs were characterized, as well as their surface morphology and internal structure. *In vitro* absorption studies were performed on the RHE model in customized Franz diffusion cells and were quantitatively and qualitatively characterized. The cytotoxicity of optimized NLCs formulations was evaluated through the *in vitro* skin irritation test on the RHE model.

All characterized NLCs presented appropriate physicochemical properties for dermal formulations and an efficient distribution and release profile of active compounds across the reconstructed skin membrane. *In vitro* skin irritation tests demonstrated that the optimized NLCs formulations were no cytotoxic.

Short CV

Fátima Pinto, PhD in Bioengineering (MIT Portugal Program, Instituto Superior Técnico), has scientific experience in the development and characterization of biodegradable lipid nanoparticles for an efficient delivery of bioactive substances. More specifically, in the encapsulation of active ingredients with commercial interest into nanostructured lipid carriers (NLCs), formulated based on the miniemulsions technology, to improve their release, stability and efficacy.

Recently integrated the project: "A predictive toxicology approach to characterize potential respiratory effects of functionalized nanocellulose fibers in a co-culture system" at Instituto Nacional de Saúde Doutor Ricardo Jorge (INSA). This project aims to evaluate the potential toxic effects of several nanocellulose (NC) samples differing in critical physicochemical properties, i.e., the characterization of biological effects triggered by NC, regarding to citotoxicity, genotoxicity, immunotoxicity and epigenetics.

https://orcid.org/0000-0002-9377-4085

INGESTnano
Cellular and Molecular Mechanisms of Toxicity of Ingested Nanomaterials
PTDC/SAU-PUB/29481/2017
https://www.facebook.com/INGESTnano

ToxApp4NanoCELFI
A PREDICTIVE TOXICOLOGY APPROACH TO CHARACTERIZE POTENTIAL RESPIRATORY EFFECTS OF FUNCTIONALIZED NANOCELLULOSE FIBRES IN A CO-CULTURE SYSTEM
(PTDC/SAU-PUB/32587/2017)

FCT Fundação para a Ciência e a Tecnologia

Nanocelluloses: production, characterization and market.

Paulo Ferreira

Department of Chemical Engineering, University of Coimbra

The presentation will address topics on the domain of nanocelluloses, namely: definitions; past, present and future for the nanoceluloses; production, properties and characterization; market; some applications in papermaking.

Short CV:

Paulo Ferreira (ORCID ID: 0000-0002-4503-6811) is an Assistant Professor with aggregation at the Department of Chemical Engineering of the University of Coimbra. He got his PhD. in 2000 and the aggregation in 2019. His main Scientific Interests are in the following areas: Pulp and Paper Science and Technology (Physico-chemical analysis, characterization, modification and functionalization of pulps, fibres, papers, paper fillers and pigments; Surface sizing, pigmentizing, nanocoating, and printability; Production, characterization and uses of nanocelulloses; Flocculation and fiber flow studies in papermaking; Paper hygroexpansivity and wet-web resistance); Particle Science and Technology (particles characterization). His Scopus H-Factor is 17, having 66 SCI papers and 78 SCOPUS papers. He supervised/s 5 PhD and 25 MSc thesis and has participated in 24 funded projects, being the principal researcher in 6 of them and the responsible at the University of Coimbra of 7 in consortium. He has near 120 publications in international conference proceedings.

INGESTnano
Cellular and Molecular Mechanisms of Toxicity of Ingested Nanomaterials
PTDC/SAU-PUB/29481/2017
https://www.facebook.com/INGESTnano

ToxApp4NanoCELFI
A PREDICTIVE TOXICOLOGY APPROACH TO CHARACTERIZE POTENTIAL RESPIRATORY EFFECTS OF FUNCTIONALIZED NANOCELLULOSE FIBRES IN A CO-CULTURE SYSTEM
(PTDC/SAU-PUB/32587/2017)

Abstracts – Session 2

Session 2 **Nanotoxicology: concepts and methodologies for toxicity evaluation of the nanomaterials.**

A predictive toxicology approach to characterize potential respiratory effects of functionalized nanocellulose fibres in a co-culture system

Célia Ventura[1], Sara Teixeira[1], Henriqueta Louro[1], Ana Filipa Lourenço[2], Paulo J.T. Ferreira[2], Maria J. Silva[1]

[1]Department of Human Genetics, Instituto Nacional de Saúde Dr. Ricardo Jorge and ToxOmics - Centre for Toxicogenomics and Human Health, NOVA Medical School, Universidade Nova de Lisboa, Lisboa;

[2]CIEPQPF, Department of Chemical Engineering, University of Coimbra, Portugal.

Cellulose nanofibrils (CNF) have an enormous potential for industrial and biomedical applications, assuming a great economic value. Because other nanofibres, e.g. carbon nanotubes (CNT), have revealed toxicity 1,2, there is a need to comprehensively evaluate the toxic potential of CNF along the value chain, before they enter the market.

This project is aimed at a safety evaluation of several CNF comparatively to CNT, in a co-culture of human-derived alveolar epithelial cells and macrophages3.

A predictive toxicology approach is used, i.e., the toxicity will be characterized alongside the specific fibre-associated mode of action, including immunotoxicity, genomic and epigenetic effects. The data obtained for two CNF synthesized from Eucalyptus gobulus but using different pre-treatments will be presented. Future work includes the use of omics-based tools adapted to the toxicity assessment of CNF and other NMs that will give some insights on cellular and molecular mechanisms underlying CNF toxicity. The overall results will be used to ensure the safety of these CNF or to allow the modification of toxic CNF in order to reduce the adverse outcomes, thereby complying with the safer-by-design approach.

Acknowledgments:

Project ToxApp4NanoCELFI (PTDC/SAU-PUB/32587/2017) is funded by the Foundation for Science and Technology (FCT/MCTES), through national funds (PIDDAC).

References:

[1] Ventura C, Sousa-Uva A, Lavinha J, Silva MJ. 2018. Environ Mol Mutagen, 59:334-362.
[2] Louro H, Pinhão M, Santos J, Tavares A, Vital N, Silva MJ. 2016. Toxicol Letters. 262: 123–13.
[3] Ventura C, Lourenço AF, Sousa-Uva A, Ferreira PJT, Silva MJ. 2018. Toxicol Letters 291:173-183.

Short CV

Maria João Silva is the PI of the *Research Group in Genetic Toxicology*, Human Genetics Department, Instituto Nacional de Saúde Dr. Ricardo Jorge, Lisbon and co-leads the research line on *Environmental and Genetic Determinants of Human Disease*, ToxOmics, NMS-FCM, Universidade Nova de Lisboa. Her main research interests comprise environmental genotoxicity, nanotoxicology, chemicals and mixtures hazard assessment, and human biomonitoring. She authored or co-authored 28 articles/book chapters since 2014. Currently she coordinates the HBM4EU Project's activities at INSA, she is the co-PI of the FCT project INGESTnano (PTDC/SAU-PUB/29481/2017) and the PI of the Project ToxApp4NanoCELFi (PTDC/SAU-PUB/32587/2017).

ORCID ID https://orcid.org/0000-0002-6060-0716.

New "omics" approaches as a tool to explore mechanistic nanotoxicology.

Célia Ventura[1], Luís Vieira[1], Catarina Silva[1], Maria João Silva[1]

[1]Department of Human Genetics, National Institute of Health Doutor Ricardo Jorge, Lisbon, and ToxOmics - Centre for Toxicogenomics and Human Health, NOVA Medical School, Universidade Nova de Lisboa, Portugal.

In the last years, genomic approaches have been applied to study the toxicity of nanomaterials with the aim of obtaining insightful information on their effects on gene expression and consequent cellular changes. Toxicogenomics expects to find unique transcriptional profiles that, besides providing evidence of the mechanistic mode of action of nanomaterials, may also be used as biomarkers for biomonitoring purposes. Moreover, several nanomaterials have been associated with epigenetic alterations, i.e., changes in the regulation of gene expression caused by DNA methylation, histone tail alterations and differential microRNA (miRNA) expression. DNA methylation is frequently studied when analysing the epigenetic regulation of gene expression and the role of miRNAs is being increasingly understood, either promoting or supressing biological pathways. Consequently, the identification of the differently expressed miRNAs in cells or tissues after exposure to a toxic can allow the recognition of its possible mechanisms of action.

An example of an epigenomic study will be presented, focusing on the exposure of epithelial alveolar cells to a multi-walled carbon nanotube (MWCNT) and asbestos (crocidolite). MWCNTs are one of the most promising products of nanotechnology with an extensive variety of applications in industry and biomedicine. Several toxicological studies have demonstrated that exposure to some MWCNTs can induce immunotoxic, cytotoxic and genotoxic effects, and nowadays they are considered as an occupational hazard. Particularly, those with a fiber-like shape similar to asbestos have raised concern about their carcinogenicity, and one (MWCNT-7) was classified in Group 2B (IARC) as a possibly human carcinogenic.

By elucidating the molecular pathways that are involved in key events of nanomaterials toxicity, the new "omics" studies are expected contribute to exclude or reduce the handling of hazardous nanomaterials in the workplace and support the implementation of regulation to protect human health.

Short CV

Researcher in the genetic toxicology group of the Human Genetics Department (HGD), National Institute of Health Doutor Ricardo Jorge (INSA). Since 1993, she works in molecular biology in the HGD, and currently participates in European and national projects on nanotoxicology. Member of ToxOmics - Center for Toxicogenomics and Human Health, NOVA University of Lisbon (UNL). Member of the Ethics Committees of the National School of Public Health, UNL, and the Portuguese Society of Human Genetics, and Ethics adviser in a Horizon 2020 project. Master Degree in Bioethics from the Portuguese Catholic University (2008), and PhD in Public Health from UNL (2019) with a thesis on the genotoxic and epigenotoxic effects of human exposure to nanofibres. Published a book on the ethics of biobanks for genetic research, (co)authored several papers in international peer-reviewed journals, and is an invited speaker in several scientific meetings. Main interest in toxicoepigenomics.

INGESTnano
Cellular and Molecular Mechanisms of Toxicity of Ingested Nanomaterials
PTDC/SAU-PUB/29481/2017
https://www.facebook.com/INGESTnano

ToxApp4NanoCELFI
A PREDICTIVE TOXICOLOGY APPROACH TO CHARACTERIZE POTENTIAL RESPIRATORY EFFECTS OF FUNCTIONALIZED NANOCELLULOSE FIBRES IN A CO-CULTURE SYSTEM
(PTDC/SAU-PUB/32587/2017)

FCT Fundação para a Ciência e a Tecnologia

In vitro assessment of nanomaterials hazard – how the experimental approach can make a difference.

Sónia Fraga, Maria João Bessa, Fátima Brandão, Luciana Moreira, Carla Costa, João Paulo Teixeira

Departamento de Saúde Ambiental, Instituto Nacional de Saúde Doutor Ricardo Jorge, Porto, Portugal; EPIUnit - Instituto de Saúde Pública da Universidade do Porto, Porto, Portugal.

In the recent years, manufacture and application of nanomaterials has developed quickly and global production of new entities is expected to increase exponentially in the coming years. The broad spectrum of nanomaterials applications, and consequently the increased risk of environmental and human exposure to these materials have raised concerns about their safety and potential adverse health effects. Despite all efforts, assessment of nanomaterials hazard has not been sufficiently profitable so far. Nanomaterials specific properties such as smaller size and larger surface area, high catalytic reactivity and distinctive physicochemical characteristics compared to their respective bulk forms offer a challenge for a reliable safety assessment using the conventional testing approaches and techniques.

An overview of the current state-of-the-art strategies for nanomaterial hazard assessment using *in vitro* approaches will be provided with a focus on inhalation toxicity testing. Practical examples will be presented to illustrate how different aspects of the experimental design (e.g. dose metrics, exposure conditions, cell type) can greatly impact the findings and conclusions of the study.

Short CV

Sónia Fraga is Auxiliary Researcher at the National Institute of Health Dr. Ricardo Jorge, Porto, Portugal. Her main research interests focus on the safety of manufactured nanomaterials. She has been involved in several projects dealing with different aspects of the health and safety hazards of nanomaterials to support risk assessment that include the occupational risks associated with manufacture of nanomaterials (ERA-NET SIINN CERASAFE) and establishment of grouping approaches to prioritise nanomaterials testing (ERA-NET SIINN NanoToxClass). Currently, she is the Principal Investigator of the NanoBioBarriers project aimed at understanding how metal nanoparticles intrinsic properties influence their interaction with the biological.

CIÊNCIA ID 0717-20FD-1212

ORCID orcid.org/0000-0001-9386-2336

INGESTnano
Cellular and Molecular Mechanisms of Toxicity of Ingested Nanomaterials
PTDC/SAU-PUB/29481/2017
https://www.facebook.com/INGESTnano

ToxApp4NanoCELFI
A PREDICTIVE TOXICOLOGY APPROACH TO CHARACTERIZE POTENTIAL RESPIRATORY EFFECTS OF FUNCTIONALIZED NANOCELLULOSE FIBRES IN A CO-CULTURE SYSTEM
(PTDC/SAU-PUB/32587/2017)

FCT Fundação para a Ciência e a Tecnologia

Cellular and molecular mechanisms of toxicity of ingested nanomaterials.

Ana C. Gramacho[1], Dora Rolo[1], Carla Martins[2,3], Ricardo Assunção[2,3], Lídia M. Gonçalves[4], Ana Bettencourt[4], Paula Alvito[2,3], Joana Pereira[1], Peter Jordan[1], Maria J.Silva[1,5], Henriqueta Louro[1,5]

[1]Department of Human Genetics, National Institute of Health Dr. Ricardo Jorge (INSA), Lisboa, Portugal.
[2]Department of Food and Nutrition, National Institute of Health Dr. Ricardo Jorge (INSA), Lisboa, Portugal
[3]CESAM - Centre for Environmental and Marine Studies, University of Aveiro, Aveiro, Portugal
[4]Research Institute for Medicines (iMed.ULisboa), Faculty of Pharmacy, Universidade de Lisboa, Portugal
[5]ToxOmics – Centre for Toxicogenomics and Human Health, NOVA Medical School, Universidade NOVA de Lisboa, Portugal

The technology based on manufactured nanomaterials (NMs) has been pointed as key enabling technology, due to its potential to improve many products and processes, namely in agriculture, food and feed industry. Many of such products, already available, have NMs such as titanium dioxide nanomaterials (TiO_2) and the oral exposure may occur either directly, through the consumption of products/pharmaceuticals containing NMs, or indirectly, through the ingestion of foods contaminated with NMs released from food-contact materials or even through concentration in the food chain due to environmental accumulation. Therefore, the gastrointestinal tract (GIT) appears to be a probable route of exposure to NMs and may lead to systemic exposure if the body barriers are surpassed.

One major concern for public health is that NMs may produce adverse outcomes (AO) such as genotoxic effects that are associated with increased risk of cancer. Although NMs have been extensively investigated in recent years, the studies have generated contradictory results, possibly due to differences in the physicochemical properties of the NMs studied and to other variables in the test systems. INSA has previously shown that NMs with the same chemistry, but differing in primary properties may yield different biological effects. Conversely, the NMs properties are context-dependent, i.e. can be affected by the surrounding matrix. These secondary features may be potentially more relevant for determining toxicological outcomes. In particular, processes like digestion may modify the NMs characteristics leading to unexpected toxicity in intestine cells.

INGESTnano project aims to investigate the nano-bio interactions of NMs, at cellular and molecular level, in the context of intestinal tract and digestion processes, to better understand their potential negative impacts on human health with special reference to organ-specific cells. TiO_2 has been selected as case-study to setup a workflow for addressing nanosafety concerns that may be in the future applied to other NMs to which GIT may be exposed. It is expected that this project will contribute to the safety evaluation of the TiO_2 ingested, by elucidating key events (KE) elicited by these NMs and linking exposure to AO.

Short CV

HL is graduated in Biochemistry (Faculty of Sciences, Lisbon University), Ph.D in Public Health (ENSP, Nova University of Lisbon) and works since 1997 in the Department of Human Genetics of the National Institute of Health Dr. Ricardo Jorge. She is a researcher in the field of genetic toxicology. She is also involved in human biomonitoring studies and her recent work involves nanotoxicology, with participation in European projects (Nanogenotox and NanoReg) as well as in national projects. She is PI of the project INGESTnano and co-PI of ToxApp4NanoCelfi (FCT funded projects).

ORCID ID: orcid.org/0000-0001-9744-7332

INGESTnano
Cellular and Molecular Mechanisms of Toxicity of Ingested Nanomaterials
PTDC/SAU-PUB/29481/2017
https://www.facebook.com/INGESTnano

ToxApp4NanoCELFI
A PREDICTIVE TOXICOLOGY APPROACH TO CHARACTERIZE POTENTIAL RESPIRATORY EFFECTS OF FUNCTIONALIZED NANOCELLULOSE FIBRES IN A CO-CULTURE SYSTEM
(PTDC/SAU-PUB/32587/2017)

FCT Fundação para a Ciência e a Tecnologia

Nanomaterials in foods and the standardized static in vitro digestion method: contributing to the study of the potential toxic effects

Martins C[1,2], Assunção R[1,2], Gramacho AC[3], Alvito P[1,2], Silva MJ[3,4], Louro H[3,4]

[1]Department of Food and Nutrition, National Institute of Health Dr. Ricardo Jorge, Lisboa, Portugal; [2]CESAM - Centre for Environmental and Marine Studies, University of Aveiro, Aveiro, Portugal; [3]Department of Human Genetics, National Institute of Health Dr. Ricardo Jorge (INSA), Lisboa, Portugal; [4]ToxOmics – Centre for Toxicogenomics and Human Health, NOVA Medical School, Universidade NOVA de Lisboa, Portugal.

The use of nanoparticles (e.g. titanium dioxide) in commercial food products to modify some properties, such as brightness and whiteness, increased in the last years and is nowadays widespread. Despite the inhalation of nanoparticles is already a topic of concern, the potential adverse health effects due to ingestion still presents gaps of knowledge. In fact, gastrointestinal tract is the first interface between the body and the external environment and consequently could represent a target organ for compounds present in food that could exert toxic effects. The *in vitro* digestion models used to simulate the human digestion may contribute to fill these gaps. A standardized *in vitro* digestion model (IVD) was developed within the INFOGEST COST Action. This method considers a three-compartment model, simulating the digestion in the mouth, stomach and small intestine, applying standardized parameters such as pH, enzymatic activity and incubation periods.

Within the scope of INGESTnano project (PTDC/SAU-PUB/29841/2017), and under physiological conditions, three TiO_2 nanomaterials (NMs) were selected to setup a workflow to address the nanosafety regarding ingested NMs. The IVD was used to simulate the human gastrointestinal digestion of the NMs and the final product was used to test and ascertain NMs toxicity, using cell lines as intestinal models.

The use of this standardized IVD model presented some challenges such as the high level of toxicity of the final digestion product for the bioassays. Several modifications to the initial protocol were investigated to overcome this issue. The results suggested that the addition of bile salts accounted for the majority of the observed toxicity.

The applicability of the harmonized *in vitro* digestion method will be discussed in view of its potential use as a tool for addressing the toxicity of ingested NMs or other food contaminants, mimicking the physiological processes.

Project funded by FCT/MCTES through national funds (PTDC/SAU PUB/29481/2017), co-funded by UID/BIM/00009/2013 (Centre for Toxicogenomics and Human Health – ToxOmics, FCT), iMed.ULisboa (Pest-UID/DTP/04138/2018) and CESAM (UID/AMB/50017/2019).

Short CV

Carla Martins works at National Institute of Health Doutor Ricardo Jorge and is a PhD student on Public Health (2015-19) and collaborator of Centre for Environmental and Marine Studies, University of Aveiro, Portugal. She started working on mycotoxins analysis, was responsible for mycotoxin quality control and analytical method validation. She develops studies on the harmonized *in vitro* digestion method and she is working on the determination of mycotoxins' biomarkers in food and biological fluids by LC-MS/MS, contributing to the exposure assessment of the Portuguese population to these toxins. She is author and co-author of several international publications, participates in national and international projects, co-supervises MSc thesis and collaborates in scientific conferences organization. Ciência ID: C419-FFAA-27E1

Using confocal microscopy for monitoring the subcellular impact of nanomaterials.

Paulo Matos

Department of Human Genetics • National Institute of Health Dr. Ricardo Jorge • Lisboa

Abstracts

The physicochemical properties of nanomaterials, such as their small size and high surface area ratio, make them ideal for many applications in industry and biomedicine. However, those same properties increase their ability to interact with cells and tissues, allowing their permeation through several biological barriers. While these abilities have been exploited in the development of novel drug-delivery systems, the widespread use of nanomaterials makes the evaluation of the potential cytotoxicity of their raw materials an important public health issue. In vivo studies are the usual gold standard when assessing compound toxicity, however, in vitro studies have also provided a lot of information regarding the toxicity and MoA of many compounds, and have proved crucial to clarify how the intrinsic and extrinsic properties of certain nanomaterials contribute to their interaction with cells an tissues. In this talk we will describe how confocal microscopy can be used in in vitro cell cultures to evaluate the subcellular impact of nanomaterials. We will point out the advantages and limitations of using confocal fluorescent microscopy in investigating how cells interact and react to the presence of different types of nanomaterial and how these can affect basic cellular functions.

Short CV

Paulo Matos has a PhD in Biochemistry by the University of Lisbon. In 2008 he earned an Associate Researcher position at INSA, within the Ciência 2007 program, and started his own independent research group. In 2013 he received a FCT Investigator 2012 development grant and moved to the University of Lisbon, where he had a five-year position as Principal Investigator and Invited Professor at the Chemistry and Biochemistry Department. In 2019 he returned to INSA with a permanent research position, integrating the Oncobiology and Signal Transduction research group. https://orcid.org/0000-0002-9379-9696.

INGESTnano
Cellular and Molecular Mechanisms of Toxicity of Ingested Nanomaterials
PTDC/SAU-PUB/29481/2017
https://www.facebook.com/INGESTnano

ToxApp4NanoCELFI
A PREDICTIVE TOXICOLOGY APPROACH TO CHARACTERIZE POTENTIAL RESPIRATORY EFFECTS OF FUNCTIONALIZED NANOCELLULOSE FIBRES IN A CO-CULTURE SYSTEM
(PTDC/SAU-PUB/32587/2017)

FCT Fundação para a Ciência e a Tecnologia

Abstracts – Session 3

Session 3 Risk assessment, standardization and regulation of nanomaterials

Adverse Outcome Pathways (AOPs) development, a tool for predictive nanotoxicology.

Dora Rolo[1] and Henriqueta Louro[1,2]

[1]National Institute of Health Dr. Ricardo Jorge (INSA), Lisbon, Portugal. [2]ToxOmics – Centre for Toxicogenomics and Human Health, NOVA Medical School, NOVA University, Lisbon, Portugal

Nanomaterials (NMs) have the potential to improve novel and useful wide applications in electronics, chemicals, environmental protection, biological medicine, food and others. Therefore, NMs rapid proliferation presents a dilemma to regulators regarding hazard identification, with increased concerns for public health.

Predictive nanotoxicology describes a multidisciplinary approach to NMs evaluation that uses a set of *in vitro* and *in silico* methods to forecast the effects on biological systems. This approach offers advantages to traditional hazard assessment methods, such as reducing the reliance on animal studies, associated costs and ethical issues. It may be used with several applications in environmental and human health risk assessment and NMs hazard identification, as well as for regulation.

The **Adverse Outcome Pathways (AOPs)** are the central element of a toxicological knowledge framework, promoted by member countries through OECD, built to support chemical risk assessment based on mechanistic reasoning. AOPs describes a logical sequence of causally linked events at different levels of biological organisation, which follows exposure and leads to an adverse health effect in humans or wildlife. The integrative analysis of the cellular and molecular mechanisms of nanotoxicity towards a definition of key events, may lead to adverse outcomes, driving a sequential line and defining an AOP landscape. Each defined AOP is available for crossing data, linking known and unknown landscapes.

Since the biological effects that relate to possible genotoxicity and increased risk of cancer due to NMs exposure are under analysis, the development and assessment of AOPs are important novel strategic tools for predictive nanotoxicology.

Short CV

PhD in Biomedicine (Universitat de Barcelona) focused on Molecular Epidemiology, vaccination impact, Antimicrobial Resistance and Virulence Mechanisms of several Gram-positive and Gram-negative pathogenic bacteria. Studies were conducted on strains isolated from asymptomatic carriers and invasive-disease patients. A high-throughput toxicity assessment of new antibiotics with anti-biofilm activity was applied in C. elegans animal model.

Development of STEAM educational activities among International "Art and Science" Projects.

Recently, at INSA, studies on nano-bio interactions of nanoparticles at cellular and molecular level, to contribute to the evaluation of risk assessment and Adverse Outcome Pathway landscape, are being developed.

ORCID ID: 0000-0003-2704-8648

INGESTnano
Cellular and Molecular Mechanisms of Toxicity of Ingested Nanomaterials
PTDC/SAU-PUB/29481/2017
https://www.facebook.com/INGESTnano

ToxApp4NanoCELFI
A PREDICTIVE TOXICOLOGY APPROACH TO CHARACTERIZE POTENTIAL RESPIRATORY EFFECTS OF FUNCTIONALIZED NANOCELLULOSE FIBRES IN A CO-CULTURE SYSTEM
(PTDC/SAU-PUB/32587/2017)

Nanomaterials, a new challenge in the workplace.

Ana Rita Alberto

Instituto de Soldadura e Qualidade (ISQ), Oeiras.

Due to the uncertainties that still exist about the real risk that nanomaterials may pose to human health and the environment, a precautionary approach should be used. The presentation will cover topics such as: hazards of the nanomaterials for the human health; methods for characterizing the exposure to nano-objects and their aggregates and agglomerates (NOAAs); risk assessment tools, recommended Occupational exposure limit values (OELs) and control of occupational risk exposure.

Short CV

MSc in Food Engineering and Nutrition from Piaget Institute, Degree in Zootechnical Engineering (Scientific- Technological Branch) from Évora University and Postgraduate Studies in Occupational Health and Safety Technician (TSSHT), level V, at Société Générale de Surveillance S.A (SGS Portugal). Health and Safety Researcher at ISQ, with up to 8 years of experience on safety consultant, in different industry and business sectors. In the last three years she has been involved in national R&D projects (NMC – New Cellulosic Materials) and European related with Nanotechnologies (LightCoce, LightMe, Purenano, PROCETS (H2020-NMBP) and NANoREG projects). Active participant in meetings/conferences related to nanosafety (e.g.: nanosafety Cluster events, SRA forums, Nanosafe conferences. Participant as an observer in the Technical Commission CT194 – Nanotechnologies.

INGESTnano
Cellular and Molecular Mechanisms of Toxicity of Ingested Nanomaterials
PTDC/SAU-PUB/29481/2017
https://www.facebook.com/INGESTnano

ToxApp4NanoCELFI
A PREDICTIVE TOXICOLOGY APPROACH TO CHARACTERIZE POTENTIAL RESPIRATORY EFFECTS OF FUNCTIONALIZED NANOCELLULOSE FIBRES IN A CO-CULTURE SYSTEM
(PTDC/SAU-PUB/32587/2017)