ABSTRACT BOOK

June 26th - 28th, 2024
Vila Real (Portugal)

SEMA | 2024

28th Spanish Environmental Mutagenesis and Genomics Society (**SEMA**) meeting

https://ojs.diffundit.com/index.php/sema/issue/view/93

sema
Sociedad Española de Mutagénesis y Genómica Ambiental

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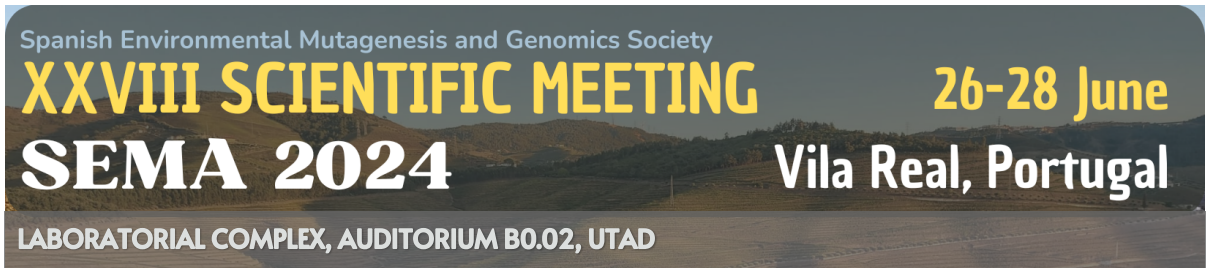
PROGRAMME



DAY 1 – JUNE 26TH WEDNESDAY

14.00-15.00 Opening Ceremony: *Teresa Roldán (SEMA president); Isabel Gaivão (organizing comitee); Francisco Peixoto (ECVA)*

SESSION 1: GENOMIC INSTABILITY, MUTAGENESIS AND DISEASE
Moderators: Alba Hernández (Universitat Autônoma de Barcelona) and **Miguel Ángel Comendador** (Universidad de Oviedo)

15:00-15:45 Keynote Lecture : Filomena Adega (DGB – UTAD -Portugal) **"Genomic instability: the players and the rules of a risky game"**

15:45-16:00 *María Sierra (UNIOVI - ES)* **Genotoxic activities of aqueous extracts from Pteridium aquilinum croziers and young fronds and their relationships with their glycoside illudane metabolites**

16:00-16:15 *Alonso Rodríguez Pescador (UNIOVI- ES)* **In vivo evaluation of ultra-small non-magnetic iron oxide nanoparticles using Drosophila melanogaster: potential applications**

16:15-16:30 *Natasha Miranda* (UTAD- PT**) Impact of high sugar diet on male fertility – study in Drosophila melanogaster as a model**

16:30-16:45 *Suzanna Correia* (UTAD- PT) **Impact of in vitro propagation on genetic stability of two Vaccinium corymbosum varieties**

16:45 - 17:15 - Coffee break

SESSION 2 : BIOMARKERS AND BIOLOGICAL MONITORING
Moderators: Amaya Azqueta (Universidad de Navarra) and **Natalia Fernández** (Universidade da Coruña)

17:15-18:00 Keynote Lecture: Carina Ladeira (Escola Superior de Tecnologia da Saúde, Lisboa, Portugal) **"Integration of effect biomarkers in human biomonitoring"**

18:00-18:15 *Carlota Lema Arranz* (UDC- ES) **Association of genetic and oxidative stress biomarkers with physical and cognitive frailty in older adults**

18:15-18:30 *Fernanda Li* (UC- PT) **Understanding sperm DNA damage: comparative analysis between donors and males in fertility consultations**

18:30-18:45 *Vanessa Sousa* (ULSSA- PT) **Comet assay reveals a high level of cumulus cells DNA damage in females sharing a phenotype of ovulatory dysfunction**

DAY 2 – JUNE 27TH THURSDAY

SESSION 3 - DNA DAMAGE, REPAIR AND PROTECTION
Moderators: María Sierra (Universidad de Oviedo) and **Vanessa Valdiglesias** (Universidade da Coruña)

9:00-9:45 Keynote Lecture: Henriqueta Louro (National Institute of Health, Lisbon, Portugal) **"Impact of genotoxicity data for tackling chemical safety challenges in regulation and health protection"**

9:45-10:00 *Marina Jordano Raya* (UCO- ES) **Influence of the orphan base on AP endonuclease activity in Base Excision Repair**

10:00-10:15 Ariadna Muñoz Fernández (UCO- ES) **Role of Base Excision Repair in temozolomide-induced DNA damage**

10:15-10:30 *Inés Grávalos* Cano (UCO- ES) **Identification of DNA repair factors involved in resistance of glioblastoma cells to temozolomide**

10:30-10:45 *Elisa Sáenz Martínez* (UNAV- ES) **Can bulky adducts be detected by employing the comet assay along with DNA repair inhbitors?**

10:45-11:00 *Sara Gonçalves* (UTAD- PT) **Unlocking Nature's Shield: Elderberry Hydrosol's Antigenotoxicological and Antioxidant Potential for Sustainable Skincare**

11:00 -11:15 *Patricia Pereira* (UA- PT) **Marine macroalgae dietary supplementation shields the white seabream (Diplodus sargus) from the chromosomal damage caused by inorganic Mercury**

11:15-11:45 - *Coffee break*

SESSION 4 - EMERGING POLLUTANTS I
Moderators: Blanca Laffon (Universidade da Coruña) and **Eduardo de la Peña** (CSIC)

11:45-12:30 Keynote Lecture: Ana Teresa Reis (National Institute of Health, Porto, Portugal)
What role do nanomaterials play in the evolving world of contaminants of emerging concern?

12:30-12:45 *Laura Rubio Lorente* (UAB- ES) **Polylactic acid nanoplastics (PLA-NPLs) induce adverse effects on an in vitro model of the human lung epithelium: the Calu-3 air-liquid interface (ALI) barrier**

12:45-13:00 *Assia Touzani* (UDC- ES) **Biocompatibility of platinum nanoparticles: study in neuronal cells and Drosophila melanogaster**

13:00-14:30 - *Lunch break*

14:30-14:45 *Jéssica Arribas Arranz* (UAB- ES) **Kinetics and toxicity of nanoplastics in ex vivo exposed human whole blood as a model to understand their impact on human health**

14:45-15:00 *Lucía Ramos Pan* (UDC- ES) **Analysis of cell cycle, apoptosis rate and DNA damage in neuronal cells exposed to differently charged gold nanoparticles**

15:00-15:15 *Mohamed Alaraby Abdalaziz* (UAB- ES) **Are bioplastics safe? Hazardous effects of polylactic acid (PLA) nanoplastics in Drosophila**

15:15-15:30 Arnau Rocabert (UAB- ES) **Changes in Drosophila microbiome caused by nanoplastics**

15:30-15:45 Laura Ruano Clemente (UAB- ES) **Long-term exposure of BEAS-2B bronchial cell line to PET nanoplastics**

SESSION 5 - GENE EXPRESSION AND EPIGENETICS
Moderators: Teresa Roldán (Universidad de Córdoba) and **Alba García** (Universitat Autônoma de Barcelona)

16:00-16:45 **Keynote Lecture** - **Carmen Jerónimo** (Instituto Português de Oncologia -Porto, Portugal) **DNA Methylation: The Rising Potential of Blood-Based Multi-Cancer Early Detection Tests**

16:45-17:00 *Carmen María Ayala Roldán* (UCO- ES) **RASSF1A methylation analysis in minimally invasive samples from Lung Cancer patients and individuals with risk factos**

17:00-22:00 *Social Event - Visit to Douro Valley*

DAY 3 – JUNE 28TH FRIDAY

SESSION 6 - EMERGING POLLUTANTS II
Moderators: Ricard Marcos (Universitat Autônoma de Barcelona) and **Óscar Herrero** (Universidad Nacional de Educación a Distancia)

9:15-9:30 *Adriana Rodríguez Garraus* (UNAV- ES) **Plastic particles derived from 3D printed objects, assessment of their cellular transforming potential**

9:30-9:45 *Javier Gutiérrez* (UAB- ES) **Carcinogenicity of micro- & nanoplastics long-term exposure**

9:45-10:00 *Michelle Morataya Reyes* (UAB- ES) **Exploring the Carcinogenic Potential of PET Nanoplastics Co-exposure with Cigarette Smoke Condensate: A Long-Term In Vitro Study Using BEAS-2B Cells**

10:00-10:15 *Juan Martin* (UAB- ES) **Hazard assessment of nanoplastics is driven by their surface-functionalization. Effects in human-derived primary endothelial cells**

10:15-10:30 *Aliro Villacorta* (UAB- ES) **Fluorescent labeling of micro/nanoplastics for biological applications with a focus on "true-to-life" tracking**

10:30-10:45 *Volodymyr Trash* (UTAD/UP- PT) **Electrochemical sensor for sucralose and its genotoxic ester. A mathematical description**

10:45 - 11:15 - Coffee break

SESSION 7: SUMMARIZE YOUR PHD THESIS (for phd students or phd´s for less than 2 years)
Moderators: Adriana Rodríguez (Universidad de Navarra) and **Antonio Guzmán** (Alexion Pharmaceuticals, Inc)

11:15-11:25 **PhD thesis summary** - **Alonso Rodríguez Pescador** (UNIOVI)

11:25-11:35 **PhD thesis summary** - **Elisa Sáenz Martínez** (UNAV)

10:35-11:45 **PhD thesis summary** - **Sara Gonçalves** (UTAD)

11:45-12:15 **Assembly of members**

12:15-12:30 **Awards Ceremony**

12:30-12:45 **Closing Ceremony**

KEYNOTE LECTURES

Genomic instability: the players and the rules of a risky game

Filomena Adega*

BioISI. Biosystems and Integrative Sciences Institute, Faculty of Sciences, University of Lisbon, 1749-016, Lisboa, Portugal
CytoGenomics Lab, Department of Genetics and Biotechnology, University of Trás-os-Montes and Alto Douro, 5000-801 Vila Real, Portugal
* filadega@utad.pt

The human current genomic architectural configuration and functionality is the sum of the most diverse sequential molecular events occurred during evolution. The events with capability for shaping genomes are based on structural and quantitative chromosomal alterations of variable dimensions, from very small to large regions that may completely change the genome structure and the cellular function. Amongst these, chromosome fusions, fissions, inversions and insertions are, perhaps, the ones with a stronger impact. Variation may occur in many different genome fields, but there are some regions that seem to be hotspots to instability. The result of such events can be then transmitted either as neutral, associated with a selective advantage that will eventually conduct to adaptation and speciation or as a potential harmful variation if occurred in fundamental cells where the outcome can be devastating. Genomic instability can thus be viewed as the strength to promote evolution but also the fragility to cause disease. Among the players of this risky game, repetitive elements such as satellite DNA sequences (the Satellitome) and transposable elements (the Mobilome), stand out as fine candidates for genomic variation and instability due to their dynamics that can impact genome architecture, genes' structure and regulation. Often considered a part of the dark matter of genomes, these sequences are now starting to be deciphered and, as players, can also be the key to understand genome adaptation, as well as normal and abnormal cellular processes, a step towards specific diseases management and even global wellness improvement.

Integration of effect biomarkers in human biomonitoring

C. Ladeira[1,2,3*]

[1] *H&TRC- Health & Technology Research Center, ESTeSL-Escola Superior de Tecnologia da Saúde, Instituto Politécnico de Lisboa, 1990-096 Lisbon, Portugal*
[2] *NOVA National School of Public Health, Public Health Research Centre, Universidade NOVA de Lisboa, Lisbon, Portugal*
[3] *Comprehensive Health Research Center (CHRC), Lisbon, Portugal*
* carina.ladeira@estesl.ipl.pt

Human biomonitoring is an important tool for assessing exposure to chemicals and their health risks. Once human internal exposure to a chemical is shown, the complementary use of effect biomarkers can help bridge health consequences by providing data on pre-clinical manifestations of disease with a probability to be prevented. Effect biomarkers that measure genetic damage are potent tools to address the carcinogenic and/or mutagenic potential of chemical exposures, increasing confidence in regulatory risk assessment decision-making processes. The micronucleus (MN) test is recognized as one of the most successful and reliable assays to assess genotoxic events, which are associated with exposures that may cause cancer. There is fair evidence of significant increase in MN frequency in patients with cancer and other chronic diseases compared with controls, substantiating its predictive value. Promising approaches, such as combined effect biomarkers open the possibility to evaluate the combined effects of complex chemical mixtures in human samples, and efforts are needed to integrate these approaches in risk assessment. Although effect biomarkers are gaining ground in human biomonitoring, new challenges are still arising. To move towards the next generation of human risk assessment is crucial to establish bridges between standard approaches of effect biomarkers and new approach methods (NAMs) and tools for increase the mechanistically-based biological plausibility in human studies, such as the adverse outcome pathways (AOPs) framework. Human epidemiological studies with biomarkers of effect play an invaluable role in identifying health effects with chemical exposures and may provide a tool for improving risk assessment and may be especially useful in the case of risk assessment of chemical mixtures.

Acknowledgements: H&TRC author gratefully acknowledges the FCT/MCTES national support through the UIDP/05608/2020(https://doi.org/10.54499/UIDP/05608/2020), UIDB/05608/2020 (https://doi.org/10.54499/UIDB/05608/2020) and, IPL/2021/PLASCOGEN_ESTeSL.

Impact of genotoxicity data for tackling chemical safety challenges in regulation and health protection

H. Louro[1,2*]

[1] *Department of Human Genetics, National Institute of Health Doutor Ricardo Jorge, I.P. (INSA), Lisbon, Portugal*
[2] *Centre for Toxicogenomics and Human Health (ToxOmics), Nova Medical School/Faculdade de Ciências Médicas, Universidade Nova de Lisboa, Lisbon, Portugal*
* henriqueta.louro@insa.min-saude.pt

The safety of chemicals used in our daily lives has gained prominence on international agendas, emerging as a cornerstone priority within the EU's chemicals strategy for sustainability, aimed at contributing to a toxic-free environment. In this context, testing guidelines have been developed to warrant the safety of chemicals in market products. Among the battery of tests recommended, genotoxicity testing gains attention due to the repercussion in the development of cancer, genetic disorders, autoimmune diseases, neurodegenerative conditions, reproductive health or premature ageing.
This work aims to elucidate the application of genotoxicity testing within various risk assessment frameworks and to highlight the potential contributions of new approach methodologies (NAMs) in this field for advancing chemical safety. Additionally, it explores the complementary roles of *in vitro* and *in vivo* evidence, amplified by the application of effect biomarkers for genotoxicity in human biomonitoring in exposed populations, in shaping policies governing chemical use. The interconnection between scientific research in genotoxicity and regulatory decision-making processes is also examined.
Overall, in the realm of chemical safety, the significance of genotoxicity data is undeniable. By leveraging these data, regulators, researchers, and stakeholders can enhance regulatory decision-making, advance toxicological understanding, support evidence-based risk communication, and foster innovation in safety assessment methodologies. Embracing the multifaceted impact of genotoxicity data is essential for promoting public health, environmental sustainability, and chemical safety in the modern world.

Acknowledgements: Thanks to all the Genetic toxicology team in the Department of Human Genetics at INSA, Lisbon, especially to the Team Leader Maria João Silva.

Funding: Co-funded by INSA and FCT through UIDB/00009/2020; UIDP/00009/2020.

What role do nanomaterials play in the evolving world of contaminants of emerging concern?

A. T. Reis[1,2,3*], F. Rosário[4], S. Fraga[1,2,3], C. Costa[1,2,3], & J. P. Teixeira[1,2,3]

[1] EPIUnit, Institute of Public Health, University of Porto, Porto, Portugal
[2] Laboratory for Integrative and Translational Research in Population Health (ITR), Porto, Portugal
[3] Environmental Health Department, National Institute of Health, Porto, Portugal
[4] CESAM—Centre for Environmental and Marine Studies & Department of Biology, University of Aveiro, Aveiro, Portugal
* ana.reis@insa.min-saude.pt

The role of nanomaterials in the evolving world of contaminants of emerging concern (CECs) is a complex and dynamic area of study. As researchers delve deeper into this field, they aim to unravel the intricate balance between nanomaterials' potential risks and benefits. These particles possess unique properties that make them valuable in a wide range of applications, from medical treatments and environmental remediation to consumer products and industrial processes. However, the very properties that make nanomaterials advantageous also raise significant concerns about their potential toxicity. Studies have shown that nanomaterials can interact with biological systems, leading to cellular uptake, oxidative stress, and genotoxicity.

Moreover, toxicity resulting from the co-exposure of nanomaterials with other contaminants, such as potentially toxic elements (PTEs) or organic chemicals, adds another layer of complexity to this issue. Research has demonstrated the intricate dynamics of co-exposure, and both synergistic and antagonistic effects have been observed, complicating risk assessments and necessitating a more comprehensive approach to studying environmental and health impacts.

This presentation will discuss the toxicological implications of nanomaterials as CECs. It will cover mechanisms of toxicity, including cellular uptake, oxidative stress, and genotoxicity, and highlight current research findings on human health effects, regulatory challenges, and the need for standardized testing protocols to manage risks associated with nanomaterials' exposure effectively.

Funding: This work was financed by FCT - Fundação para a Ciência e a Tecnologia in the framework of the project MixIng-Tox, 2022.03491.PTDC (https://doi.org/10.54499/2022.03491.PTDC). A.T. Reis thanks the financial support of FCT through individual Grant SFRH/BPD/122112/2016. The authors also thank FCT/MCTES for the financial support to EPIUnit (UIDB/04750/2020) and ITR (LA/P/0064/2020).

DNA Methylation: The Rising Potential of Blood-Based Multi-Cancer Early Detection Tests

Carmen Jerónimo*

Head, Cancer Biology & Epigenetics Group
Director, IPO Porto Research Center
Portuguese Oncology Institute of Porto (IPO Porto)
Invited Full Professor at the Department of Pathology and Molecular Genetics
ICBAS-School of Medicine & Biomedical Sciences, University of Porto
Porto, Portugal
* carmenjeronimo@ipoporto.min-saude.pt & cljeronimo@icbas.up.pt

DNA methylation, the most well-studied epigenetic mechanism, involves the addition of a methyl group to the 5-carbon of cytosines within CpG dinucleotides. While most CpG dinucleotides are scattered across gene coding regions and repetitive sequences, clusters of CpGs, known as CpG islands, are primarily found in gene promoters and first exons. In normal cells, CpG islands tend to be unmethylated, whereas coding and repetitive sequences are typically methylated. However, this methylation pattern is reversed in cancer cells: promoters become hypermethylated, leading to the silencing of tumor suppressor genes, and there is global hypomethylation, which results in genomic instability. This aberrant methylation often occurs very early in the carcinogenic process, making DNA methylation an attractive biomarker for early cancer detection. In the ongoing battle against cancer, one of society's foremost goals is to detect cancer at its earliest stages significantly increasing the prospects of successful treatment and reducing the need for subsequent therapies that often come with severe side effects and additional health complications. Moreover, the pursuit of biomarkers amenable to simultaneously detect the most common cancers has lately gained the attention of several researchers worldwide.

In my talk, I will focus on the major recent findings of my research team in our quest to develop a cell-free DNA (cfDNA) methylation-based test to simultaneously detect the four major cancers: breast (BrC), lung (LC), colorectal (CRC), and prostate (PCa). I will also discuss the current challenges associated with implementing this test in clinical settings.

Acknowledgements: Nursing Staff, IPO Porto's Patients &Healthy Donors**.**

Funding: Portuguese Oncology Institute of Porto Research Center (CI-IPOP-74-2016), UCIPredict (TRANSCAN3/0001/2021).

SESSIONS

01. Genomic instability, mutagenesis and disease

Genotoxic activities of aqueous extracts from *Pteridium aquilinum* croziers and young fronds and their relationships with their glycoside illudane metabolites

A. Cué[1], D. López[1], J. San Juan[1], M. Martín[1], L. Rodríguez[2], I. Feito[2], M. I. Sierra[3], J. Majada[4], H. Fernández[3], & L. M. Sierra[1,5,6] *

[1] Dpt. of Functional Biology (Genetics Area). University of Oviedo, C/ Julián Clavería s/n, 33006, Oviedo, Spain
[2] Programa Forestal. Servicio Regional de Investigación y Desarrollo Agroalimentario del Principado de Asturias (SERIDA), Finca Experimental La Mata, La Mata s/n, 33825 Grado, Spain
[3] Dpt. Biology of Organisms and Systems (Plant Fisiology Area). University of Oviedo, C/ Catedrático Rodrigo Uría s/n, 33071 Oviedo, Spain
[4] CETEMAS, Pumarabule, 33936 Siero, Spain
[5] Oncology University Institute of Asturias (IUOPA), University of Oviedo, Spain
[6] Institute of Sanitary Research of Principality of Asturias. Avda de Hospital Universitario s/n, 33011, Oviedo, Spain
* lmsierra@uniovi.es

The common fern *Pteridium aquilinun*, apart from being a biodiversity problem, because of its invasive properties, is also a health risk for animals and humans. Its consumption as food causes several diseases in animals, that can end up in death. In humans, its consumption, direct or indirectly, might be the origin of different types of digestive tumours. It is known for years that the carcinogenic components of Pteridium are the glycoside illudanes ptaquiloside, caudatoside and ptesculentoside, and their unstable dienone metabolites. Other more stable metabolites like the corresponding pterosines were described to be non-carcinogenic. After determining the *in vivo* genotoxic activities of aqueous extracts of Pteridium samples, collected in different parts of Asturias, in *Drosophila melanogaster*, we have studied their relationships with the respective metabolite content.

Samples were collected in several localizations in Asturias, at different altitudes, and along the National and Natural Parks. Aqueous extracts were obtained by vertical agitation for 2.5 hours of the lyophilized plant in milliQ water. The genotoxic activity was estimated with the eye SMART assay of *D. melanogaster*. Metabolite content was determined with ultra-high precision liquid chromatography linked with mass spectrometry (UHPLC-MS/M).

Results of genotoxic activity show clear differences among the different samples, with a negative correlation with the altitude. The metabolites detected in this analysis were the ptaquiloside and the three pterosines, B, A and G (from ptaquiloside, caudatoside and ptesculentoside, respectively). The genotoxicity data were analysed together with the different metabolite levels. Positive, statistically significant correlations were found with pterosine B and pterosine A levels. Since these metabolites were described as non-genotoxic, their contents could be acting as indicators of the levels of ptaquiloside and caudatoside, and of their corresponding dienones, that could not be measured. However, further information on pterosine genotoxicity is being gathered.

Funding: Ministerio de Ciencia e Innovación (Spain). Projects of Ecological and Digital Transition, Reference TED2021-131270B-I00.

In vivo evaluation of ultra-small non-magnetic iron oxide nanoparticles using *Drosophila melanogaster*: potential applications

Alonso Rodríguez Pescador[1,2,3], Lucía Gutiérrez Romero[3,4], Elisa Blanco-González[3,4], María Montes-Bayón[3,4], & L. María Sierra[1,2,3]

[1] Department of Functional Biology (Genetic Area). University of Oviedo, C/ Julián Clavería s/n, 33006, Oviedo, Spain
[2] Oncology University Institute of Asturias (IUOPA)
[3] Institute of Sanitary Research of Principality of Asturias. Avda de Hospital Universitario s/n, 33011, Oviedo, Spain
[4] Department of Physical and Analytical Chemistry, Faculty of Chemistry. University of Oviedo. C/ Julian Clavería 8, 33006 Oviedo, Spain
* rodriguezalonso@uniovi.es

Iron oxide nanoparticles (IONPs) are used in many different applications, including biomedical ones. Among this type of nanoparticles, the non-magnetic, ultra-small IONPs, composed of a ferrihydrite core covered with tartaric and adipic acids (FeAT-NPs), show special characteristics. In studies with human cultured cells, FeAT-NPs showed suitable properties for their use as an oral anemia treatment, and as a transporter of platinum drugs. However, *in vivo* evaluation is needed in order to assess their effectiveness and/or toxicity. These studies were carried out using *Drosophila melanogaster* as model organism.

The potential toxicity and genotoxicity of FeAT-NPs were evaluated, in two different conditions of the nucleotide excision repair system (NER; active and inactive), with the eye SMART assay, that detects induction of somatic mutation and recombination. To evaluate the effectiveness of FeAT-NPs as an anemia treatment, iron uptake was analyzed measuring Fe levels in Drosophila larvae using inductively coupled plasma mass spectrometry (ICP-MS). To test their role as drug carriers, FeAT-NPs were conjugated with the prodrug cisplatin (IV), and they were evaluated with the eye SMART assay. Treatments with cisplatin (II) and its prodrug, cisplatin (IV), were performed to compare the transport effectiveness. The SMART assay was carried out with surface treatments. The effectiveness of the transport was determined by ICP-MS, quantifying the levels of Pt on DNA.

Results show that FeAT-NPs are efficiently taken up by Drosophila larvae and increase their Fe levels. They show weak genotoxic activity at the highest tested concentrations, without any toxicity. Their conjugation with cisplatin (IV) prodrug show less genotoxicity than that of cisplatin (II) or cisplatin (IV), in both NER conditions. Levels of Pt on DNA are being determined. These results altogether show that FeAT-NPs might be a suitable oral anemia treatment, but their usefulness as chemotherapy treatment needs further analysis.

Impact of high sugar diet on male fertility – study in *Drosophila melanogaster* as a model

Natasha Miranda[1,2,3*], Ana Martins-Bessa[2,3,4], & Isabel Gaivão[2,3,5]

[1] Student of 2nd year of PhD in Comparative Molecular Genetics, University of Trás-os-Montes, and Alto Douro, Vila Real, Portugal
[2] CECAV - Animal and Veterinary Research Center, Department of Veterinary Sciences, University of Trás-os-Montes, and Alto Douro, Vila Real, Portugal
[3] AL4AnimalS - Associate Laboratory for Animal and Veterinary Sciences,
[4] Veterinary Sciences Department University of Trás-os-Montes and Alto Douro, Vila Real, Portugal
[5] Department of Genetics and Biotechnology, University of Trás-os-Montes, and Alto Douro, Vila Real, Portugal
** natasha.gomesdemiranda@gmail.com*

The reduction in sperm quality parameters in humans with diabetes mellitus (DM), such as concentration, viability, and morphology, shows that DM can influence the reproductive capacity of individuals who have the disease which, in some cases, can also be acquired by their descendants. *Drosophila* is a promising experimental model for studying diabetes, as it presents several metabolic changes, such as signaling pathways like mammals. The aim of this research was to analyze the effects of a high sucrose diet on male reproductive functions, morphological and behavioral changes and DNA damage in *D. melanogaster (Oregon K strain)*. We evaluated the effects of sucrose on longevity (average and maximum), negative geotaxis, spatial exploration, offspring size, genotoxicity (basal DNA damage), and sperm morphology. Young males (2 to 3 days old) were divided into five groups, fed with sucrose concentrations of 0%, 5%, 10% (control), 15% and 20% (weight/volume of standard medium) and placed in this diet for 48 to 72 h before mating. The results were analyzed in the first filial generation, where diets with 15% and 20% sucrose showed a 17% decline in average longevity (114 vs 95 days), an impact on locomotion with an 8% increase in displacement compared to the control group and a decline in exploration of 9% (43 vs 39 cm^2), as well as a drop in the number of descendants of 47% (277 vs 146 descendants). Concentrations of 15% and 20% showed a significant increase in DNA damage at the level of neuroblasts with average arbitrary units (AU) of 158 and 170 AU, respectively, compared to the control group which had 26 AU. The data highlights the significant influence that increased sucrose consumption has on *D. melanogaster*, affecting its fertility, survival, and genetic damage. Future work will analyze the role of epigenetic mechanisms in diabetes.

Acknowledgments: This work was supported by the project UIDB/00772/2020 (https://doi.org/10.54499/UIDB/00772/2020) funded by the Portuguese Foundation for Science and Technology (FCT).

Impact of in vitro propagation on genetic stability of two *Vaccinium corymbosum* varieties

S. Correia[1*], F. Leal[2,3,4], & M. Matos[2,3,4]

[1] Student of the 2nd year of the master's degree in Comparative and Technological Molecular Genetics, UTAD, Quinta de Prados, 5000-801 Vila Real, Portugal
[2] Department of Genetics and Biotechnology, University of Trás-os-Montes and Alto Douro, Vila Real, Portugal
[3] CITAB- UTAD, Quinta de Prados, 5000-801 Vila Real, Portugal
[4]Inov4Agro Associated Laboratory, UTAD, Quinta de Prados, 5000-801 Vila Real, Portugal
** auniversitariasuzanna@gmail.com*

Blueberries (*Vaccinium corymbosum*) are recognized for their beneficial properties, due to their high content of antioxidants such as anthocyanins, flavonoids, and phenolic acids. These bioactive compounds play a crucial role in neutralizing free radicals, thus mitigating oxidative stress and inflammation, both implicated in several chronic diseases, including cardiovascular disease (CVD), neurodegenerative disorders and cancer. With the increasing interest in functional foods and natural health products, blueberries stand out for their health-promoting properties.

In recent years, advances in biotechnology have enabled the rapid propagation of plants through in vitro culture techniques. Micropropagation, in particular, offers the possibility of cloning plants efficiently, thereby facilitating the preservation and dissemination of desirable traits. However, the in vitro culture process may inadvertently introduce genetic variations in plants, potentially impacting their molecular characteristics and, consequently, their metabolic profile, content of bioactive compounds, and gene expression.

In this context, the present study aims to investigate the impact of in vitro propagation on the genetic stability of two commercially important blueberry varieties, namely *Duke* and *Bluecrop*. Using molecular markers, namely inter simple sequence repeats (ISSRs), we try to find genetic variation induced by in vitro culture.

Comparing amplification patterns, genomic stability was verified in samples obtained by micropropagation, when compared to the field plant. The most significant effects were observed in plants obtained in the presence of growth regulators. These findings underscore the importance of maintaining genomic integrity for blueberry offering insights into mitigating genetic instability in micropropagation practices.

Acknowledgements: This work is supported by National Funds from FCT - Portuguese Foundation for Science and Technology, under the project UIDB/04033/2020 (https://doi.org/10.54499/UIDB/04033/2020). The plant material was kindly provided by Paulo Silva, Lda.

SESSIONS

02. Biomarkers and biological monitoring

Association of genetic and oxidative stress biomarkers with physical and cognitive frailty in older adults

C. Lema-Arranz[1,2*], A. Hemadeh[1,2], N. Cibeira[3], R. López-López[3], N. Fernández-Bertólez[2,4], J. Méndez[4], M. Sánchez-Flores[1], A. Maseda[3], J. C. Millán-Calenti[3], J. L. Rodríguez-Villamil[3], E. Pásaro[1,2], V. Valdiglesias[2,4], L. Lorenzo-López[3], & B. Laffon[1,2]

[1]Universidade da Coruña, Grupo DICOMOSA, CICA—Centro Interdisciplinar de Química e Bioloxía, Departamento de Psicología, A Coruña, Spain
[2] Instituto de Investigación Biomédica de A Coruña (INIBIC), Complexo Hospitalario Universitario de A Coruña (CHUAC), Sergas, A Coruña, Spain
[3] Universidade da Coruña, Gerontology and Geriatrics Research Group, Instituto de Investigación Biomédica de A Coruña (INIBIC), Complexo Hospitalario Universitario de A Coruña (CHUAC), Sergas, A Coruña, Spain
[4] Universidade da Coruña, Grupo NanoToxGen, CICA—Centro Interdisciplinar de Química e Bioloxía, Departamento de Biología, A Coruña, Spain
** carlota.lema.arranz@udc.es*

The concept of frailty - as opposite of full health or 'robustness' - has been proposed as a more accurate measure of biological age, due to the high inter-individual variability in ageing manifestations. Frailty is a multidimensional syndrome characterised by the loss of functions and reserves (energy, physical capacity, cognition and health) and related to an increased risk of negative health outcomes, including illness, falls, disability, institutionalisation and death. Due to the reversible characteristics of frailty, its early identification is critical and therefore knowledge on the factors involved in its physiopathology is crucial. Oxidative stress acts as a key factor in the ageing process, being involved in age-dependent diseases. Besides, genomic instability is considered a primary hallmark of ageing. Therefore, the aim of this study was to determine the possible association of genetic and oxidative stress biomarkers with frailty status (physical and cognitive) in older adults. Thus, a cross-sectional study was conducted in a population of 154 Spanish older adults (aged 65 and over) classified according to their physical (phenotype criteria) and cognitive frailty status. Primary and oxidative DNA damage were evaluated in whole blood by the standard and fpg-modified comet assay, respectively. Total antioxidant capacity, 8-hydroxy-2'-deoxyguanosine (8OHdG) and cell-free DNA were also analysed in serum samples. Results showed significantly higher total antioxidant capacity and lower cell-free DNA in both physical frail and cognitive frail groups as compared with the healthy one, but no differences were observed for 8OH-dG and for primary and oxidative DNA damage. Further research with additional biomarkers is needed to ascertain the role of genomic instability and oxidative stress in frailty development.

Funding: Ministry of Science and Innovation: MCIN/AEI/10.13039/501100011033 (Grant PID2020-113788RB-I00), Xunta de Galicia (ED431B 2022/16) and European Grouping of Territorial Cooperation – Galicia North of Portugal (IACOBUS Program).

Understanding sperm DNA damage:comparative analysis between donors and males in fertility consultations

F. Li[1*], R. Arantes[2], Z. Gomes[3], O. Moutinho[3], I. Gaivão[4], & R. Pinto-Leite[2]

[1] Faculty of Medicine, University of Coimbra, Coimbra, Portugal
[2] Genetics and Andrology Laboratory, Local Health Unit of Trás-os-Montes and Alto Douro, Vial Real, Portugal
[3] Department of Woman and Child, Local Health Unit of Trás-os-Montes and Alto Douro, Vial Real, Portugal
[4] Department of Genetics and Biotechnology and Veterinary and Animal Research Centre, University of Trás-os-Montes and Alto Douro,Vila Real, Portugal
* fernanda_carina_ly@hotmail.com

The assessment of sperm genomic integrity has become important in the field of Assisted Reproductive Technologies (ART). Recognizing its influence on fertility and reproductive outcomes, research has been conducted to try to establish reference values for DNA damage to facilitate early identification of infertility risks and improve understanding of the factors that influence the genomic integrity of spermatozoa. This work aimed to evaluate DNA damage levels (basal and oxidative) in sperm from two groups: control (A) (unselected male population with unknown reproductive health) and men undergoing fertility evaluation (B).

Thirteen samples from Group A and 9 normozoospermic samples from Group B were evaluated (individuals reporting no smoking/alcohol consumption, chronic diseases, medications, or multivitamin supplement intake). The participants' age and body mass index (BMI) were recorded. The samples were collected after a sexual abstinence period of 2-3 days and subjected to DNA damage assessment using the Alkaline Comet Assay.

We found that, regarding age, group B (36.33 years) had an age slightly higher than that associated with increased DNA damage (35 years) and significantly higher than the age of group A (25.77 years) ($p=0.001$). Concerning BMI, it was slightly higher (25.41 *vs.* 24.67 kg/m^2), although without statistical significance. Important were the results we obtained from DNA damage: our findings indicate that males undergoing fertility consultations present twice as much DNA damage in their sperm cells compared to male donors, regardless of age and BMI ($p<0.05$, $R^2=0.5$).

Despite being preliminary and constrained by the small sample size, these data highlight the significance of sperm genomic integrity in evaluating male fertility. This holds particular relevance in cases where the underlying cause of infertility is unclear or when conventional assessment techniques yield unsatisfactory results. Thus, assessing DNA damage in sperm emerges as a valuable addition to the arsenal of tools available in ART.

Funding: This work was supported by the projects UIDB/00772/2020 (Doi:10.54499/UIDB/00772/2020), and LA/P/0059/2020, funded by the Portuguese Foundation for Science and Technology (FCT).

Comet assay reveals a high level of cumulus cells DNA damage in females sharing a phenotype of ovulatory dysfunction

V. Sousa[1, 2, 3*†], B. Rodrigues[1, 2, 3†], E. Vale-Fernandes[2, 3, 4], D. Sousa[4], R. Brandão[4], C. Leal[4], I. Gaivão[5], F. Esteves[3, 6, 7], S. Costa[3, 6, 7], J. Pires[3, 6, 7], J.P. Teixeira[3, 6, 7], A. J. A. Nogueira[8], & P. Jorge[1, 2, 3]

[1] Laboratório de Genética Molecular, Serviço de Genética Laboratorial, Clínica de Genética e Patologia, Unidade Local de Saúde de Santo António (ULSSA), Porto, Portugal
[2] UMIB - Unit for Multidisciplinary Research in Biomedicine, ICBAS - School of Medicine and Biomedical Sciences, University of Porto, Porto, Portugal
[3] ITR - Laboratory for Integrative and Translational Research in Population Health, Porto, Portugal
[4] Centre for Medically Assisted Procreation/ Public Gamete Bank, Gynaecology Department, Centro Materno-Infantil do Norte Dr. Albino Aroso (CMIN), Unidade Local de Saúde de Santo António (ULSSA), Porto, Portugal
[5] CECAV - Veterinary and Animal Research Centre and Department of Genetics and Biotechnology, University of Trás-os-Montes and Alto Douro (UTAD), Vila Real, Portugal
[6] Environmental Health Department, National Institute of Health Dr Ricardo Jorge, Porto, Portugal
[7] EPIUnit - Instituto de Saúde Pública, Universidade do Porto, Porto, Portugal
[8] CESAM - Center for Environmental and Marine Studies, Department of Biology, University of Aveiro, Aveiro, Portugal
* vanessa_sousa4d@hotmail.com
† *equal contribution*

Ovulatory dysfunction phenotypes include disturbances in menstrual cycles and hormonal impairments. Females with ovulatory dysfunction show irregular or absent ovulation commonly causing infertility. Cumulus cells (CC) are used to explore biomarkers for this reproductive health burden due to their proximity to the oocyte and their role in its development and maturation. CC DNA damage has been associated with oocyte status; however, the existing studies present contradictory results.

Our aim was to assess DNA damage in infertile females undergoing intracytoplasmic sperm injection (ICSI). The alkaline comet assay was performed on whole blood and CC from 42 females. Among them, 20 exhibited ovulatory dysfunctions, while the remaining 22 experienced mostly male-related infertility. The age range was matched to ensure similar hormonal secretory patterns. The CCs were obtained after oocyte denudation during the common ICSI procedure. Significant differences were obtained in the DNA damage levels of the two tissues ($p < 0.001$). Furthermore, the levels of DNA damage were higher in CC when compared to blood and were significantly different between females with ovulatory dysfunction and those experiencing male-related infertility ($p = 0.034$). An interesting positive correlation was obtained between CC DNA damage levels and the number of oocytes with two pronuclei (2PN) ($p = 0.026$). Following other studies, we speculate that the level of blood DNA damage reflects that of heterogeneous cell types, is dependent on several exogenous factors, and therefore does not mirror the level of damage of CC. The differences in the DNA damage levels obtained between the two tissues and the higher levels observed in CC prompt us to explore its potential utility in addressing fertility. The positive correlation with the number of oocytes in 2 PN suggests that CC damage level underlies a success of fertilization in females with an ovulatory dysfunction phenotype.

Funding: EXPL/BIA-REP/0423/2021, SFRH/BD/13398/2018 & COVID/BD/153204/2023, UID/AMB/50017/2019, UIDB/00215/2020, UIDP/00215/ 2020, LA/P/0064/2020.

SESSIONS

03. DNA damage, repair and protection

Influence of the orphan base on AP endonuclease activity in Base Excision Repair

M. Jordano-Raya[1,2,3], R. R. Ariza[1,2,3], T. Roldán-Arjona[1,2,3], & D. Córdoba-Cañero[1,2,3]

[1] Department of Genetics, University of Córdoba, Córdoba, Spain
[2] Maimonides Biomedical Research Institute of Cordoba (IMIBIC), Córdoba, Spain
[3] Reina Sofía University Hospital, Spain
* b52joram@uco.es

Abasic (apurinic/apyrimidinic, AP) sites are ubiquitous DNA lesions that can arise from the spontaneous loss of a nitrogenous base or as intermediates during the Base Excision Repair (BER) pathway. AP sites can be processed by either AP endonucleases or AP lyases, but the relative roles of these two types of enzymes are not well understood. We hypothesized that the sequence flanking the AP site and the orphan base opposite the lesion may determine the enzyme responsible for its processing and the repair efficiency. We compared the AP incision activities of human, bacterial, and plant AP endonucleases using DNA substrates containing an abasic site opposite guanine (G), adenine (A), thymine (T), or cytosine (C). We observed no preference for the opposite base in the major human AP endonuclease, APE1. However, a strong effect of the orphan base was observed for the plant (*Arabidopsis* ARP) and the bacterial (*Escherichia coli* Exo III) orthologues, which showed their lowest efficiency on AP sites opposite C. Using structural and homology information we identified differentially conserved residues in APE1, ARP, and Exo III. Mutations of these residues resulted in significant changes in AP site processing, depending on the orphan base. Our results suggest that opposite-base specificity is an ancestral feature of AP endonucleases that may have been lost in the metazoan lineage. The lack of specificity in APE1 may be related to its ability to efficiently cleave AP sites on both single-stranded (ssDNA) and double-stranded DNA (dsDNA). In contrast, Arabidopsis ARP exhibits an inability to incise AP sites on ssDNA. We have identified specific residues responsible for discriminating between these two types of DNA substrates. Our study highlights the functional differences between human, plant, and bacterial AP endonucleases and provides new insights into the evolution of DNA repair pathways.

Funding: This research has been funded by MICINN (PID2019-109967GB-I00 and PID2022-140458NB-I00). M. Jordano-Raya is the recipient of a PhD FPU Fellowship from MIU.

Role of Base Excision Repair in temozolomide-induced DNA damage

A. Muñoz-Fernández[1,2,3*], C. Ayala-Roldán[1,2,3], S. Conde-Maestre [1,2,3], T. Morales-Ruiz[1,2,3], A. L. Gomez [4,5], R. R. Ariza [1,2,3], T. Roldán-Arjona [1,2,3], & M. I. Martínez-Macías[1,2,3]

[1] Maimonides Biomedical Research Institute of Cordoba (IMIBIC), Córdoba, Spain
[2] Department of Genetics, University of Córdoba, Córdoba, Spain
[3] Reina Sofía University Hospital, Córdoba, Spain
[4] Instituto de Salud y Ambiente del Litoral (ISAL. UNL-CONICET), Facultad de Bioquímica y Ciencias Biológicas, Universidad Nacional del Litoral, Argentina
[5] Cátedra de Patología Humana, Facultad de Bioquímica y Ciencias Biológicas, Universidad Nacional del Litoral, Argentina
** e-mail: b62mufea@uco.es*

Since the efficiency of chemotherapy in cancer treatment is counteracted by the action of DNA repair pathways, it is often necessary to combine anticancer drugs with specific DNA repair inhibitors. Temozolomide (TMZ) is a DNA alkylating agent used in the treatment of glioblastoma (GBM), an aggressive form of brain tumour with a low survival rate due in great part to resistance to TMZ. The main lesion induced in DNA by TMZ is N7-meG, harmless by itself but prone to generate abasic (AP) sites that are highly cytotoxic and mutagenic. AP sites are repaired through the Base Excision Repair (BER) pathway initiated either by AP endonucleases or by AP lyases. Recently, it has been shown that in *Arabidopsis thaliana,* the AP sites generated from N7-meG are processed through an AP lyase/DNA phosphatase pathway mediated by the lyase FPG and the phosphatase ZDP. In human cells, the homologous protein of ZDP is the polynucleotide kinase 3'-phosphatase, PNKP. In the present work, we aimed to study the role of PNKP in the repair of AP sites induced by TMZ and, in doing so, highlight its relevance to the cellular sensitivity to this agent. We have used TMZ-resistant and sensitive GBM cell lines to specifically block the activity of PNKP by a specific inhibitor (PNKPi) and to deplete its expression by small interference RNA (siRNA). Using such approach, we examined the impact of PNKP in TMZ sensitivity (by clonogenic and survival assays), in the repair of TMZ-dependent breaks (by alkaline comet assays) and in cellular apoptosis (by flow cytometry). Our data show that the depletion of PNKP activity (by PNKPi or siRNA) sensitizes GMB cells to TMZ treatment, impairs the repair of TMZ-induced breaks and increases cellular apoptosis. Our results may help to identify novel therapeutical targets in TMZ- treated tumours.

Funding: Ministerio de Ciencia e Innovación (PID2019-109967GB-I00; PID2022-140458NB-I00), European Union's Horizon 2020 research and innovation programme under the Marie Skłodowska-Curie grant agreement No 847468. A. M.-F. is supported by the "Programa Investigo", funding by Ministerio de Trabajo y Economía Social y la Unión Europea. (Expediente 2021-C23.I01.P03.S0020-0000004).

Identification of DNA repair factors involved in resistance of glioblastoma cells to temozolomide

I. Grávalos-Cano[1,2,3*], T. Morales-Ruiz [1,2,3], R. R. Ariza [1,2,3], T. Roldán-Arjona [1,2,3], & M. I. Martínez-Macías [1,2,3]

[1] Department of Genetics, University of Córdoba, Córdoba, Spain
[2] Maimonides Biomedical Research Institute of Cordoba (IMIBIC), Córdoba, Spain
[3] Reina Sofía University Hospital, Córdoba, Spain
** ge2grcai@uco.es*

Glioblastoma (GBM) is an aggressive brain tumour with poor survival rate due, in great part, to resistance to temozolomide (TMZ), the primary chemotherapeutic agent used adjunctively with radiotherapy after surgery. TMZ is an alkylating agent that induces three primary DNA lesions: N7-methylguanine (70%), N3-methyladenine (10%), and O6-methylguanine (7%). Resistance to TMZ has been associated with high expression of DNA repair proteins involved in the repair of these lesions, such as MGMT (O6-meG DNA methyltransferase) or MPG (N-Methylpurine DNA Glycosylase). However, elevated levels of such proteins only explain some cases of TMZ-resistant tumours, suggesting that resistance to this alkylating agent is mediated by several DNA repair pathways. Our aim is to understand the mechanisms used by glioblastoma cells to repair DNA damage induced by TMZ. To achieve this goal, we carried out a transcriptional study of DNA repair genes using PCR arrays in GBM cell lines (A172, T98G, LN229, LN18, U373 and CCF-STTG1) which exhibit different levels of TMZ sensitivity. Out of the 84 genes studied, only DDB2 (DNA damage binding protein 2) showed differences in expression between sensitive and resistant GBM cells. Subsequently, we validated the array results through RT-qPCR and Western blot analysis. Additionally, studies were conducted to examine the expression of DDB2 in GBM cells in response to TMZ treatment. Moreover, we examined whether the expression of DDB2 is regulated by epigenetic mechanisms such as DNA methylation. Our results suggest that DDB2 play a role in the response to TMZ in GBM cells, opening new perspectives to understand the resistance to alkylating agents in tumours.

Funding: Ministerio de Ciencia e Innovación (PID2019-109967GB-I00; PID2022-140458NB-I00), European Union's Horizon 2020 research and innovation programme under the Marie Skłodowska-Curie grant agreement No 847468. I. G.-C. is the recipient of a PhD fellowship (PREDOC_00426) from Junta de Andalucía (Consejería de Transformación Económica, Industria, Conocimiento y Universidades).

Can bulky adducts be detected by employing the comet assay along with DNA repair inhibitors?

E. Saenz-Martinez[1*], A. López de Cerain[1], & A. Azqueta[1]

[1] Department of Pharmaceutical Sciences, School of Pharmacy and Nutrition, University of Navarra, Pamplona, Spain
** esaenzm@alumni.unav.es*

The standard alkaline comet assay is a simple and economical genotoxic test widely used in genetic toxicology for the detection of strand breaks and alkali labile sites in the DNA. With several modifications it can also detect altered bases such as oxidized and alkylated bases and cross-links. However, it cannot detect bulky adducts, an important DNA lesion in which a chemical is bond to the DNA. Since DNA bulky adducts are mainly repaired by nucleotide excision repair (NER), the comet assay has been modified using NER inhibitors, such as the combination of hydroxyurea (HU) and cytosine arabinoside (Ara-C) or aphidicolin (APC), that blocks reparation process and causes incision breaks intermediates to accumulate. These modifications have been used without a proper validation study.

An internal validation study has been carried out using TK6 cells treated with a compound which causes DNA bulky adducts, six genotoxic agents with different mechanisms or two cytotoxicity controls, together with the DNA repair inhibitors. The MTS assay and comet assay were performed to determine cytotoxicity and DNA damage respectively.

Although more data is needed for a final conclusion, the use of HU/Ara-C or APC in combination with the comet assay increases the sensitivity of the comet assay for the detection of DNA damage, despite being nonspecific for the detection of DNA bulky adducts. Moreover, these results challenge the concept that different lesions in the DNA are repaired by different mechanisms or the specificity of the NER inhibitors.

Funding: Spanish Ministry of Sciences, Innovation and Universities (BIOGENSA2, PID2020-115348RB-100; FPU21/03187).

Unlocking Nature's Shield: Elderberry Hydrosol's Antigenotoxicological and Antioxidant Potential for Sustainable Skincare

Sara Gonçalves[1,2,3*], Francisco Peixoto[4], & Isabel Gaivão[1,2]

[1] CECAV and Department of Genetics and Biotechnology, University of Trás-os-Montes and Alto Douro, 5000-801 Vila Real, Portugal
[2] Associate Laboratory for Animal and Veterinary Sciences (AL4AnimalS), University of Trás-os-Montes and Alto Douro, 5000-801 Vila Real, Portugal
[3] Academic Clinical Center of Trás-os-Montes and Alto Douro - Professor Doctor Nuno Grande – CACTMAD, University of Trás-os-Montes and Alto Douro, 5000-801 Vila Real, Portugal
[4] Centro de Química de Vila Real (CQVR), Biology and Environment Department, University of Trás-os-Montes and Alto Douro, 5000-801 Vila Real, Portugal
* sgoncalves@utad.pt

Elderberry (*Sambucus nigra*) has garnered attention for its rich content of beneficial phytochemicals and its diverse applications in traditional medicine. Recently, elderberry hydrosol has emerged as a promising ingredient and holds potential as a valuable resource for various applications, including cosmetics, aromatherapy, and possibly as a functional ingredient in dietary supplements. This study aims to assess the antigenotoxic and antioxidant properties of elderberry hydrosol. The methodology involved domestic hydrodistillation to obtain elderberry hydrosol. Four concentrations (1%, 5%, 10%, and 15% w/v) of elderberry hydrosol were used to assess the antigenotoxic potential in human peripheral blood mononuclear cells after exposure to hydrogen peroxide. We evaluated DNA damage in these cells using the Comet assay to determine its protective effects against DNA damage. Antioxidant assays, including ABTS (2,2′-azinobis(3-ethylbenzothiazoline-6-sulfonic acid)) and DPPH (2,2-Diphenyl-1-picrylhydrazyl), were employed to elucidate its antioxidative potential. The hydrodistillation process produced elderberry hydrosol, demonstrating a significant transfer of aromatic and therapeutic compounds, emphasizing its effectiveness and environmentally friendly characteristics. The genotoxicity assessments revealed the hydrosol's ability to protect against DNA damage at all concentrations tested. Notably, the 1% treatment exhibited the least DNA damage, with 3.7% DNA in the tail, indicating optimal efficacy at this concentration. Furthermore, the ABTS and DPPH assays showed concentration-dependent responses, with higher concentrations correlating with increased antioxidant potency. The highest antioxidant activity was observed at a hydrosol concentration of 15%, corresponding to 0.4 µmol and 0.03 µmol TEAC for ABTS and DPPH, respectively. Elderberry hydrosol is a promising and versatile component for skincare formulations. It provides various benefits and effectively counters DNA damage induced by ROS. Its efficacy and eco-friendly nature make it a valuable addition to natural cosmetic products. Nevertheless, continuous research into its bioactive constituents and mechanisms is essential to harness its potential and promote sustainable skincare practices.

Acknowledgements: The authors would like to thank Dinis Diogo and Guy Gonçalves for providing the ingredients analysed in this research and assisting in the hydrodistillation. The authors would like to thank Tiago Ferreira for helping with the statistical analysis.

Funding: This work was supported by the project UIDB/00772/2020 (Doi:10.54499/UIDB/00772/2020), funded by the Portuguese Foundation for Science and Technology (FCT).

Marine macroalgae dietary supplementation shields the white seabream (*Diplodus sargus*) from the chromosomal damage caused by inorganic mercury

Ana Neto[1], Rute Cesário[2], Fátima Brandão[1], Vitória Pereira[1], Ana Marques[1], Marisa Barata[3], Pedro Pousão-Ferreira[3], Helena Abreu[4], Mário Pacheco[1], & Patrícia Pereira[1*]

[1] Centre for Environmental and Marine Studies (CESAM) and Department of Biology, University of Aveiro, Aveiro 3810-193, Portugal
[2] Centro de Química Estrutural, Institute of Molecular Sciences and Department of Chemical Engineering, Instituto Superior Técnico, Universidade de Lisboa, Portugal
[3] Portuguese Institute for Sea and Atmosphere (IPMA) - Aquaculture Research Station, 8700-005 Olhão, Portugal
[4] ALGAplus, Lda., PCI - Creative Science Park, 3830-352 Ílhavo, Portugal
** pkpereira@ua.pt*

Marine macroalgae have been studied as human health promoters. Despite the numerous advantages of marine macroalgae supplementation, its benefits to improve fish health condition remains elusive. This study aimed to investigate the genoprotection afforded by a marine macroalgae enriched diet to the white seabream (*Diplodus sargus*) when exposed to inorganic mercury (iHg), as well as its benefits on the hematological dynamics. For this purpose, fish were fed during 3 months with a marine macroalgae-enriched feed (Ma; total incorporation of 5%, with *Ulva rigida*, *Fucus vesiculosus* and *Gracilaria gracilis*, equitably represented), while non-supplemented fish were fed with a standard diet (S). Then, both dietary background groups were exposed to waterborne iHg (2 µg L^{-1}) for 7 days (E7) (groups MaHg and SHg), followed by a post-exposure period of 14 days (PE14) to address recovery. Control fish, unexposed to iHg, were maintained over the experiment (MaC and SC). At E7 and PE14, fish of the different groups (MaC, SC, MaHg, SHg) were sacrificed and blood was collected for the determination of total Hg levels, assessment of chromosomal integrity (as erythrocytic nuclear abnormality assay; ENA) and hematological dynamics (as erythrocytic maturity index; EMI) in peripheral erythrocytes. Fish that were fed with a macroalgae-enriched diet accumulated significantly lower levels of Hg than those under a standard diet, both at E7 and PE14. Accordingly, the ENA results pointed out a decrease of iHg genotoxicity at the group feed with the macroalgae-enriched diet at E7. Additionally, the EMI revealed an increase of class 2 erythrocytes in fish fed with a macroalgae-enriched diet upon exposure to iHg (E7), suggesting an increased cell lifespan. Overall, results are promising by revealing, for the first time, the genoprotection of a macroalgae dietary supplementation against the chromosomal damage induced by iHg in fish erythrocytes.

Acknowledgements: Thanks are due to FCT/MCTES for the financial support to CESAM (UIDP/50017/2020+UIDB/50017/2020), through National funds. Pereira P. and Cesário R. are funded by National funds (OE) through FCT (Fundação para a Ciência e a Tecnologia, I.P.), under the Scientific Employment Stimulus–Individual Call (https://doi.org/10.54499/CEECIND/01144/2017/CP1459/CT0009 and https://doi.org/10.54499/2021.03267.CEECIND/CP1651/CT0010, respectively).

SESSIONS

04. Emerging pollutants I

Polylactic acid nanoplastics (PLA-NPLs) induce adverse effects on an *in vitro* model of the human lung epithelium: the Calu-3 air-liquid interface (ALI) barrier

Alba García-Rodríguez[1*], Javier Gutiérrez[1], Aliro Villacorta[1,2], Jéssica Arribas Arranz[1], Iris Romero-Andrada[3], Alicia Lacoma[3], Ricard Marcos[1] Alba Hernández[1], & Laura Rubio[1]

[1] Group of Mutagenesis, Department of Genetics and Microbiology, Universitat Autònoma de Barcelona, Cerdanyola del Vallès, Spain
[2] Facultad de Recursos Naturales Renovables, Universidad Arturo Prat, Iquique, Chile
[3] Institut d'Investigació Germans Trias i Pujol, Badalona, Spain
** laura.rubio@uab.cat*

The expected increments in the production/use of bioplastics, as an alternative to petroleum-based plastics, require a deep understanding of their potential environmental and health hazards, mainly as nanoplastics (NPLs). Since one important exposure route of NPLs is inhalation, here we aimed at studying the fate and effects of true-to-life polylactic acid nanoplastics (PLA-NPLs), using the *in vitro* Calu-3 model of bronchial epithelium under air-liquid interphase exposure conditions. To determine the health risk of PLA-NPLs in a more realistic scenario, both acute (24 h) and long-term (1 and 2 weeks) exposures were performed. Results indicate that PLA-NPLs internalized easily in the barrier (~10% at 24 h and ~40% after 2 weeks), affecting the expression of tight-junctions formation (~50% less *vs* control) and the mucus secretion (~50% more *vs* control). Interestingly, significant genotoxic effects (DNA breaks) were detected by using the comet assay, long-term effects being more marked than acute (7.01 *vs* 4.54% of DNA damage). When an array of cellular proteins including cytokines, chemokines, and growth factors were used, a significant over-expression was mainly found in long-term exposures (~20 proteins *vs* 5 proteins after the acute exposure). Overall, these results describe the potential hazards posed by PLA-NPLs, under relevant long-term exposure scenarios, highlighting the advantages of the model used to study bronchial epithelium tissue damage and signaling endpoints related to inflammation.

Biocompatibility of platinum nanoparticles: study in neuronal cells and *Drosophila melanogaster*

Assia Touzani[1,2*], Lucía Ramos-Pan[1,2], Natalia Fernández-Bertólez[1,2], Luisa Martínez[1], María Luz Díaz-Prado[3], Blanca Laffon[2,4], & Vanessa Valdiglesias[1,2]

[1] Universidade da Coruña, Grupo NanoToxGen, Centro Interdisciplinar de Química e Bioloxía—CICA, Departamento de Bioloxía, Facultade de Ciencias, Campus A Zapateira s/n, 15071 A Coruña, Spain
[2] Instituto de Investigación Biomédica de A Coruña (INIBIC), Complexo Hospitalario Universitario de A Coruña (CHUAC), Sergas, As Xubias, 15006 A Coruña, Spain
[3] Universidade da Coruña, Grupo NEUROVER, Departamento de Bioloxía, Facultade de Ciencias, Campus A Zapateira s/n, 15071 A Coruña, Spain
[4] Universidade da Coruña, Grupo DICOMOSA, Centro Interdisciplinar de Química e Bioloxía—CICA, Departamento de Psicoloxía, Facultade de Ciencias da Educación, Campus Elviña s/n, 15071 A Coruña, Spain
** assia.touzani@udc.es*

Platinum nanoparticles (PtNP) have attracted increasing interest in the biomedical field due to their unique properties, offering a wide range of applications ranging from diagnosis to therapy. However, despite their promising potential, the toxic effects of PtNP and their cellular and molecular impact remain largely unknown. With this in mind, the present study aimed to discard possible *in vitro* and *in vivo* effects of PtNP on the SH-SY5Y neuroblastoma cell line and on the model organism *Drosophila melanogaster*. For the *in vitro* evaluation, MTT test, comet assay and challenge-comet assay were employed to evaluate cytotoxic and genotoxic effects. The *in vivo* study with *D. melanogaster* included analysis of morphological alterations in both larvae and adult individuals chronically exposed, as well as behavioural evaluation by the crawling assay in third-instar larvae. Results obtained revealed decreases in viability of SH-SY5Y cells exposed to PtNP but limited to the highest doses tested. No effects on viability, DNA damage or DNA repair were observed at biological relevant doses according to MTT, comet assay and challenge-comet assay analyses, respectively. After chronic oral exposure to PtNP, our study revealed no significant difference in size in *D. melanogaster* individuals, neither adults or larvae. However, behavioural alterations were observed for exposed larvae that showed dose-dependent significant decreases in their mobility. These initial findings point to a high biocompatibility of PtNP, but some hints of *in vivo* effects under the experimental conditions tested support the need of further investigation before PtNP can be safely used in biomedical applications.

Funding: This research was funded by Ministry of Science and Innovation: MCIN/AEI/10.13039/501100011033 (Grant PID2020-114908GA-I00) and Xunta de Galicia (ED431B 2022/16).

Kinetics and toxicity of nanoplastics in *ex vivo* exposed human whole blood as a model to understand their impact on human health

J. Arribas Arranz[1*], A. Villacorta[1,2], L. Rubio[1], A. Garcia-Rodriguez[1], M. Sanchez[3], M. Llorca[3], M. Farre[3], J. F. Ferrer[3], R. Marcos[1], & A. Hernández[1]

[1] *Group of Mutagenesis, Department of Genetics and Microbiology, Faculty of Biosciences, Universitat Autònoma de Barcelona, Cerdanyola del Vallès, Spain*
[2] *Facultad de Recursos Naturales Renovables, Universidad Arturo Prat, Iquique, Chile*
[3] *Institute of Environmental Assessment and Water Research CSIC, Barcelona, Spain*
* *jessica.arribas@uab.cat*

The ubiquitous presence of micro/nanoplastics (MNPLs) in the environment is considered of great health concern. Since MNPLs can cross both the intestinal and pulmonary barriers, their presence in the blood compartment is expected; consequently, understanding the interactions between MNPLs and human blood is required. In this study, to simulate more adequately the real exposure conditions, exposure was done on whole blood, and five different MNPLs: three polystyrene NPLs of around 50 nm (aminated, carboxylated, and pristine forms), together with two true-to-life MNPLs from polyethylene terephthalate (PET) and polylactic acid (PLA) of around 150 nm were used. Internalization was determined in white blood cells (WBCs) by confocal microscopy, once the different cell types (monocytes, PMNs, and lymphocytes), were sorted by flow cytometry. Reactive oxygen species (ROS) induction was determined in WBCs as well as the cytokine release in plasma. In addition, hemolysis, coagulation, and platelet activation were also determined. Results showed a differential uptake between WBC types with a higher internalization in monocytes. Regarding ROS, lymphocytes were those producing higher levels and with different NPLs. Cytokine releases were observed after exposures, with higher effects after PLA- and PS-NH2-NPL exposure. Hemolysis induction was observed after PS- and PS-NCOOH-NPL exposure, but no effects on platelet functionality were observed. Finally, it must be stated that this is the first study determining the effects of different NPL types on human whole blood and evaluating bloodstream toxicity.

Funding: This project (PlasticHeal) has received funding from the European Union's Horizon 2020 research and innovation program under grant agreement No 965196. A. Villacorta was supported by Ph.D. fellowships from the National Agency for Research and Development (ANID), CONICYT PFCHA/DOCTORADO BECAS CHILE / 2020 − 72210237.

Analysis of cell cycle, apoptosis rate and DNA damage in neuronal cells exposed to differently charged gold nanoparticles

L. Ramos-Pan[1,2], A. Touzani[1,2], N. Fernández-Bertólez[1,2], J. Méndez[1], M. Paz[3], J. Mosquera[3], B. Laffon[2,4], & V. Valdiglesias[1,2*]

[1] Universidade da Coruña, Grupo NanoToxGen, Centro Interdisciplinar de Química e Bioloxía – CICA, Departamento de Biología, A Coruña, Spain
[2] Instituto de Investigación Biomédica de A Coruña (INIBIC), A Coruña, Spain
[3] Universidade da Coruña, Grupo Nanoself, Centro Interdisciplinar de Química e Bioloxía – CICA, Departamento de Química, A Coruña, Spain
[4] Universidade da Coruña, Grupo DICOMOSA, Centro Interdisciplinar de Química e Bioloxía – CICA, Departamento de Psicología, A Coruña, Spain
** vanessa.valdiglesias@udc.es*

Gold nanoparticles (AuNP) are widely used for numerous applications in different fields, and in the last years they have gained significant attention in biomedicine. Due to their small size and particular physical-chemical properties, AuNP stand out for their potential application in neurological disorders, since they can cross the blood-brain barrier. In this context, it is relevant to discard any possible adverse effects on nervous system cells once they are introduced into the body. On this basis, the main objective of this work was to assess the potential cytotoxic and genotoxic effects of differently charged AuNP (i.e. anionic, cationic and neutral) at biologically relevant concentrations on human neuronal cells (SH-SY5Y) treated for 3 and 24 h. Potential alterations of cell cycle as well as apoptosis induction were assessed by flow cytometry as indicative of cytotoxicity, whereas genotoxicity was analyzed by comet assay and γH2AX analysis. Slight cell cycle alterations were observed for all AuNP whereas increased percentage of apoptosis cells were found just for neutral AuNP after 24 h of treatment. Furthermore, the three tested AuNP showed a low genotoxic potential, inducing very slight primary DNA damage in comet assay and low, although significant, increases of double strand breaks evaluated by γH2AX assay. These results provide a better understanding of AuNP biological behaviour and are satisfactory for their possible use in nervous system-targeted applications.

Funding: Ministry of Science and Innovation: MCIN/AEI/10.13039/501100011033 (Grant PID2020-114908GA-I00) and Xunta de Galicia (ED431B 2022/16).

Are bioplastics safe? Hazardous effects of polylactic acid (PLA) nanoplastics in *Drosophila*

M. Alaraby[1,2*], D. Abass[1,2], A. Hernández[1], & R. Marcos[1]

[1]Group of Mutagenesis, Department of Genetics and Microbiology, Universitat Autònoma de Barcelona, Cerdanyola del Vallès, Spain
[2]Zoology Department, Sohag University (82524), Sohag, Egypt
* mohamed.alaraby@uab.cat

Background: While the production of bioplastics increases continuously, there is a gap of information on the hazardous impact of their degradation products (micro/nanoplastics, MNPLs).

Aim: To understand the potential health risks associated with the exposure to MNPLs of bioplastics.

Methods: To address this issue, *Drosophila melanogaster* as a versatile terrestrial *in vivo* model was employed, and polylactic acid nanoplastics (PLA-NPLs), as a proxy for bioplastics, were tested as a material model. A wide battery of approaches has been applied in this study including internalization, gene expression, oxidative stress, and genotoxicity.

Results: The harmful effects were determined in larvae exposed for 4 days to different concentrations (25, 100, and 400 µg/mL) of 463.9 ± 129.4 nm PLA-NPLs. Transmission electron microscopy (TEM) and scanning electron microscope (SEM) approaches permitted the detection of PLA-NPLs in the midgut lumen of *Drosophila* larvae, interacting with symbiotic bacteria. Enzymatic vacuoles were observed as potential carriers, collecting PLA-NPLs and enabling the crossing of the peritrophic membrane, finally internalizing into enterocytes. Although no toxic effects were observed in the egg-to-adult survival, the cell uptake of PLA-NPLs causes cytological disturbances and the formation of large vacuoles. The translocation across the intestinal barrier was demonstrated by their presence in the hemolymph. Furthermore, PLA-NPL exposure triggered intestinal damage, oxidative stress, DNA damage, and inflammation responses, as evaluated via a wide set of marker genes. Collectively, these structural and molecular interferences caused by PLA-NPLs generated high levels of oxidative stress and DNA damage in the hemocytes of *Drosophila* larvae.

Conclusions: The observed effects point out the need for further studies aiming to deepen the health risks posed by bioplastics before considering their uses as a safe plastic alternative to the petroleum-based plastics.

Changes in Drosophila microbiome caused by nanoplastics

Arnau Rocabert[1*], Hannes Van Goethem[1], Jordi Cabrera[2], Juan Martin[1], Mohammed Alaraby[1], Alba Garcia-Rodrigez[1], Jaime Martinez-Urtaza[2], Ricard Marcos[1], & Alba Hernandez[1]

[1] Group of Mutagenesis, Department of Genetics and Microbiology, Faculty of Biosciencies, Universitat Autònoma de Barcelona, Cerdanyola del Vallès, Spain
[2] Group de Genomics, Bioinformatics & Evolutive Biology (GBBE), Department of Genetics and Microbiology,Faculty of Biosciencies, Universitat Autònoma de Barcelona, Cerdanyola del Vallès, Spain
** 1531681@uab.cat*

There is a growing interest in studying the human gut microbiome with the objective of comprehending the relations between microorganism, the host and exogenous stressors like environmental pollutants. However, the study of the human microbiome is a big step to overcome due to the vast amount of different organism found (e.g., bacteria, viruses, fungi, etc.) and the difficulties/disparities found when sampling (biopsy vs stool samples). For this reason, experiments with animals of lower complexity have been proposed as a working step towards preliminary understanding of the gastrointestinal microbiome and its modulatory interaction with exogenous contaminants. One of the animals is *Drosophila melanogaster*, the fruit fly, a model organism used in different fields due to many characteristics that makes working with it very easy.

In this work, the objective is to study the effect that different micro and nanoplastics have on the gut microbiome of *D. melanogaster* larvae, as well as adult flies. For this reason, larvae as well as flies were treated with MNPLs, PET, polystyrene and PLA were used at 200 µg/mL for 1 week as well as zinc as a control, then the microbiome was extracted using ZymoBIOMICS extraction kits and sequenced using MinION Nanopore technology.

Results show differences between larvae and adult fly samples as well as effects on the alpha and beta diversity of the microbiome, even though the high variability of *Drosophila* microbiome presents a challenge. Further investigations will be conducted in order to overcome this problem as well as using some other organisms for testing the effects, such as mice.

Funding: This work was partially supported by the EU Project PLASTICHEAL (nº 965196) HORIZON 2020, the Spanish Ministry of Science and Innovation (PID2020-11689, RB-C43), the Generalitat de Catalunya (2021-SGR00731), and the ICREA-Academia programme to AH. AR and JMP are funded by the Generalitat de Catalunya (FI-SDUR 2023).

Long-term exposure of BEAS-2B bronchial cell line to PET nanoplastics

Laura Ruano[1*†], Javier Gutierrez[1†], Laura Rubio[1], Raquel Egea[1], Alba Garcia[1], Ricard Marcos[1], & Alba Hernández[1]

[1] Department of Genetics and Microbiology, Faculty of Biosciences, Universitat Autònoma de Barcelona, Cerdanyola del Vallès, Barcelona, Spain
** Laura.RuanoC@autonoma.cat*
[†] equal contribution

Nanoplastics (NPLs) are ubiquitous emerging pollutants with great facility to cross through body barriers and bioaccumulate in different tissues. Inhalation is a major way of human exposure, and little is known of NPLs hazard effects in lung. The aim of this study is to evaluate if long-term exposures to NPLs are able to induce carcinogenic effects, simulating a more realistic scenario. To achieve this goal, BEAS-2B bronchial cells were treated with 50 µg/ml of polyethylene terephthalate (PET) NPL for 30 weeks. In order to assess potential harmful effects, genotoxicity, carcinogenicity and gene expression were evaluated through a battery of *in vitro* assays and transcriptomic analyses at three time points: 24 h, 15 weeks, and 30 weeks of exposure. Results showed no genotoxic nor carcinogenic effects after 24 h or 15 weeks, however, after 30 weeks of exposure genotoxic damage, invasive ability and anchorage-independent growth potential were increased. Transcriptomic analyses showed significant alterations in terms of gene expression: differentially expressed genes (DEGs) increased progressively along the time of exposure and gene set enrichment analysis (GSEA) revealed altered pathways after treatment, including epithelial to mesenchymal transition, myogenesis, inflammation pathways, xenobiotic metabolism, cholesterol metabolism and estrogen response. In addition, the identification of potential biomarker candidates was carried out, and 8 genes were selected as candidate targets to detect transformation progression. In conclusion, long-term exposure of BEAS-2B cells to PET-NPLs, and potentially other NPLs, can induce genotoxic, carcinogenic and transcriptomic alterations.

Funding: This project (PlasticHeal) has received funding from the European Union's Horizon 2020 research and innovation programme under grant agreement No 965196. This work was partially supported by the Spanish Ministry of Science and Innovation [PID2020-116789, RB-C43], and the Generalitat de Catalunya (2021-SGR-00731).


SESSIONS
05. Gene expression and epigenetics

RASSF1A methylation analysis in minimally invasive samples from lung cancer patients and individuals with risk factors

Carmen Ayala-Roldán[1,2,3]*, Rocío Santiago-Domínguez[1,2,3], Litzy Gisella Bermúdez Liscano[1,4], Camila Bernal Forigua[1,4], Jesús Romero Simmonds[4], María Isabel Martínez-Macías[1,2,3], Bernabé Jurado Gamez[2,3,5], Laura Caballero Ballesteros[2,3], María del Sol Arenas de Larriva[2,3], Rafael R. Ariza[1,2,3], Teresa Roldán-Arjona[1,2,3], Adriana Patricia Rojas Moreno[1,2,3,4], & Teresa Morales-Ruiz[1,2,3]

[1] Department of Genetics. University of Cordoba, Córdoba, Spain
[2] Maimonides Biomedical Research Institute of Cordoba (IMIBIC), Córdoba, Spain
[3] Reina Sofia University Hospital, Córdoba, Spain
[4] Institute of Human Genetics, School of Medicine, Pontificia Universidad Javeriana, Bogotá, Colombia
[5] Department of Medical and Surgical Sciences. University of Cordoba, Córdoba, Spain
* b82ayroc@uco.es

Ras association domain family isoform A (*RASSF1A*) is a tumour suppressor gene. *RASSF1A* exerts its functions through its scaffolding properties, allowing the assembly of complexes involved in various signaling pathways. The RASSF1A protein contains three major domains: the C1/DAG domain, the Ras association domain (RA) and a Sav/RASSF/Hpo interaction domain (SARAH). The RA domain mediates the interaction of RASSF1A with members of the Ras GTPase families, inhibiting their oncogenic function and thereby affecting the processes of cell proliferation, differentiation, morphogenesis and apoptosis in response to extracellular signals. *RASSF1A* inactivation is common in several human cancers. The main mechanism associated with *RASSF1A* inactivation is gene silencing through DNA methylation. In particular, in lung cancer, the most aggressive tumours with the worst prognosis are those in which K-RAS is mutated and the *RASSF1A* promoter is hypermethylated.

In this work, we aimed to analyze the *RASSF1A* methylation status in minimally invasive samples (blood plasma) from lung cancer patients and individuals with risk factors (smoking and chronic obstructive pulmonary disease, COPD). Samples were classified into four groups: 1) control group without risk factors (healthy); 2) smokers with risk factors (Smokers); 3) COPD risk factor group (COPD) and 4) lung cancer group (LuCa). DNA was extracted, bisulfite-modified, and quantitative methylation specific PCR (qMSP) was performed to determine the methylation status of the gene. In addition, we analyzed the *RASSF1A* methylation status in samples from lung cancer cell lines (A549, H23, PC9 and H292).
We detected *RASSF1A* methylation in plasma samples from 38% LuCa, 10% COPD, 12.5% Smokers and 12.5% healthy subjects. These findings were confirmed in lung cancer cell lines. These preliminary data suggest that methylation of *RASSF1A* may be a useful epigenetic biomarker for diagnosis of lung cancer using minimally invasive samples.

Funding: University of Cordoba, under Grant Submodalidad 2.6. UCOIMPULSA to TMR**,** BPIN 2020000100363 SGR-MINCIENCIAS-Colombia to APRM.

SESSIONS

06. Emerging pollutants II

Plastic particles derived from 3D printed objects, assessment of their cellular transforming potential

Adriana Rodríguez-Garraus[1,2*], Mari Venäläinen[1], Jussi Lyyränen[1], Hanna Pulli[1], Apostolos Salmatonidis[3], Davide Lotti[4], Josefa Domenech[1], José F. Fernández[5], Jesús Guzmán-Mínguez[5], Alberto Katsumiti[6], Isabel Rodríguez-Llopis[6], Socorro Vázquez-Campos[3], Marie Carriere[7], & Julia Catalán[1]

[1] Finnish Institute of Occupational Health, 00250 Helsinki, Finland
[2] University of Navarra, 31008 Pamplona Navarra, Spain
[3] LEITAT Technological Center, 08225 Terrassa, Spain
[4] LATI Industria Termoplasti SPA, 21040 Vedano Olona, Italy
[5] CSIC Spanish National Research Council, 28049 Madrid, Spain
[6] GAIKER Technology Centre, Basque Research and Technology Alliance (BRTA), 48170 Zamudio, Spain
*[7] Univ. Grenoble-Alpes, CEA, CNRS, SyMMES-CIBEST, 38000 Grenoble, France**
* arodriguez.53@unav.es*

Humans are consistently exposed to micro- and nano-plastics (MNPs) resulting from the degradation of plastic waste, affecting their health. MPs accumulation in tissues and organs raises concerns about cancer induction and validated carcinogenic studies conducted with rodents present economic and ethical dilemmas. Conversely, in vitro cell transformation assays (CTAs) provide information regarding in vivo simulation of initiation and promotion stages of carcinogenesis.

The aim of this study was to assess the cell-transforming potential of MNPs generated during the degradation of 3D printed objects at the end of their lifecycle, using the validated Bhas-42 CTA.

Polycarbonate particles with and without single walled carbon nanotubes (PC-CNT and PC, respectively), and polypropylene particles with and without silver nanoparticles (PP-Ag and PP, respectively) were obtained by cryomilling 3D printed objects. The obtained particles (~0.30 µm) were dispersed following the NanoGenotox protocol and evaluated through the Bhas-42 CTA (OECD guidance 231). In the initiation assay, cells were exposed to the particles for four days and in the promotion assay, for 14 days. The concentrations tested were from 6.25 to 100 µg/ml. Cell growth, internalization and gene expression were assessed in parallel cultures at day 4 of exposure.

All the materials induced concentration-dependent cell growth decrease in the initiation assays, especially PP-Ag. The cell growth decrease was less pronounced in the promotion assays. All the materials were internalized into the cells, nevertheless none of them induced transformed foci in the initiation or promotion assays. Gene expression is still being analysed.

Funding: This project has received funding from the European Union's Horizon 2020 research and innovation program under grant agreement No. 862419 (SAbyNA).

Carcinogenicity of micro- & nanoplastics long-term exposure

J. Gutiérrez[1]*, J. Domenech[2], I. Barguilla[3], M. Morataya[1], L. Rubio[1], B. Guyot[3], R. Marcos[1], V. Maguer-Satta[3], J. Catalán[2], & A. Hernández[1]

[1] Group of Mutagenesis, Department of Genetics and Microbiology, Universitat Autònoma de Barcelona, Cerdanyola del Vallès (Barcelona) 08193, Spain
[2] Finnish Institute of Occupational Health, Box 40, Työterveyslaitos, 00032 Helsinki, Finland
[3] Department of Cancer Initiation and Tumor Cell Identity, Centre de Recherche en Cancerologie, Lyon, France
** Javier.Gutierrez.Garcia@uab.cat*

Micro- & nano-plastics (MNPLs) are considered emergent pollutants widely spread over all environmental compartments. There is evidence that humans can internalize these MNPLs through inhalation and ingestion and that the small size of the plastic particles may allow for absorption, systemic biodistribution and bioaccumulation. Despite of the fact that their potential biological effects are being intensively evaluated, their potential health effects in humans remain poorly understood. One of the most underdeveloped areas of study is the determination of the effects induced by MNPLs under chronic scenarios of exposure, being carcinogenicity the most relevant in terms of risk. In this context, the present talk will focus on presenting the current science on MNPLs carcinogenic potential, giving special attention to the approaches developed and results obtained in the frame of the large-scale EU Project PlasticHeal (www.plasticheal.eu/en). Together with the available literature, the set of obtained data supports a potential carcinogenic risk associated to MNPLs long-term exposure. On this basis of evidence, the need of more studies becomes evident. Key research questions and remaining knowledge gaps will therefore be discussed in benefit of future research and the full assessment of MNPLs carcinogenic risk.

Funding: This project (PlasticHeal) has received funding from the European Union's Horizon 2020 research and innovation programme under grant agreement No 965196. This work was partially supported by the Spanish Ministry of Science and Innovation [PID2020-116789, RB-C43], and the Generalitat de Catalunya (2021-SGR-00731).

Exploring the Carcinogenic Potential of PET Nanoplastics Co-exposure with Cigarette Smoke Condensate: A Long-Term In Vitro Study Using BEAS-2B Cells

M. Morataya-Reyes[1]*, A. Villacorta[1,2], J. Arribas[1], J. Gutierrez[1], L. Rubio[1], R. Marcos[1], & A. Hernández[1]

[1] Group of Mutagenesis, Department of Genetics and Microbiology, Universitat Autònoma de Barcelona, Cerdanyola del Vallès, Spain
[2] Facultad de Recursos Naturales Renovables, Universidad Arturo Prat, Iquique, Chile
* herlem.morataya@uab.cat

Air pollution stands as a primary contributor to global disease and premature mortality, responsible for over 7 million premature deaths annually. Among its contemporary concerns, micro and nanoplastics (MNPLs) have garnered significant attention due to their detection within human lungs, with polypropylene (PP) and polyethylene terephthalate (PET) fibers emerging as predominant species. Despite existing studies examining the impacts of certain MNPLs in animal and *in-vitro* models, they typically focus on short-term exposures, failing to capture the reality of persistent, cumulative exposure and bioaccumulation. Addressing this critical gap, our research simulates real-life scenarios through a novel long-term exposure study utilizing BEAS-2B human bronchial epithelial cells. In a previous assessment, BEAS-2B cells were continuously exposed to true-to-life polyethylene terephthalate nanoparticles (NPET), with evaluations conducted at various intervals to assess cell transformation biomarkers. At the conclusion of a 30-week exposure period, NPET-exposed cells exhibited elevated genotoxic DNA damage and an increased anchorage-independent growth ability compared to control cells. These findings, coupled with observations of PET-induced tumor promotion in the OECD's Bhas-42 cell transformation assay (CTA), denote the induction of a "prone to transformation progress (PTP)" phenotype in the BEAS-2B cell line under prolonged NPET exposure. To accelerate this transformation process, PTP cells were subjected to 4-week co-exposure to NPET and cigarette smoke condensate (CSC), followed by a reassessment of biomarkers. The NPET-CSC co-exposed cells exhibited significantly greater total DNA damage compared to non-exposed cells and those solely exposed to CSC. While colony size remained consistent across treatments, the number of colonies and their migratory and invasive capabilities were notably enhanced in PET and NPET-CSC exposed cells. Utilizing cell transformation biomarkers, our study demonstrates the oncogenic potential of long-term NPET exposure in BEAS-2B cells, emphasizing the relevance of investigating MNPL effects under conditions mirroring contemporary human exposures.

Funding: This work was partially supported by the EU Horizon 2020 programme (965196, PlasticHeal) and the Research and Universities Department from the Generalitat de Catalunya (FI-AGAUR 2022).

Hazard assessment of nanoplastics is driven by their surface-functionalization. Effects in human-derived primary endothelial cells

Joan Martín-Pérez[1*], Aliro Villacorta[1,2], Gooya Banaei[1], Michelle Morataya-Reyes[1], Alireza Tavakolpournegari[1], Ricard Marcos[1], Alba Hernández[1], & Alba García-Rodriguez[1]

[1]Group of Mutagenesis, Department of Genetics and Microbiology, Faculty of Biosciences, Universitat Autònoma de Barcelona, Cerdanyola del Vallès, 08193, Spain
[2]Facultad de Recursos Naturales Renovables, Universidad Arturo Prat, Iquique, Chile
* Juan.Martin.Perez@uab.cat

In the process of breaking down plastic waste into micro/nanoplastics (MNPLs), various aspects of their physical and chemical characteristics, such as surface properties (charge, functionalization, biocorona, etc.), may undergo changes, potentially influencing their biological impacts. This study specifically investigates the surface functionalization of MNPLs to ascertain its potential direct effects on toxicokinetic and toxicodynamic interactions within human umbilical vein endothelial cells (HUVECs), across different exposure durations.

Pristine polystyrene nanoplastics (PS-P NPLs), alongside their carboxylated (PS-C NPLs) and aminated (PS-A NPLs) counterparts, each approximately 50 nm in size, were subjected to an extensive array of toxicological assessments. These evaluations encompassed analyses of cell viability, internalization within cells, generation of intracellular reactive oxygen species (iROS), and assessment of genotoxicity. The experiments were conducted at a concentration of 100 µg/mL, chosen to ensure a high rate of internalization across all treatments while maintaining a sub-toxic dose. Results indicate that all types of PS NPLs are internalized by HUVECs, with the dynamics of internalization varying depending on the particle's specific functionalization. Both PS-P and PS-C NPLs induce modifications in cell morphology, increasing inner complexity/granularity. However, only PS-A NPLs demonstrated a reduction in cell viability. Intracellular ROS generation was triggered by all three types of PS NPLs, albeit at different time intervals. Genotoxic damage was observed with all PS NPLs after short exposures (2 h), except for PS-C NPLs at 24 h. Overall, this study underscores the surface-dependent toxicological effects of PSNPLs on HUVEC cells, emphasizing the importance of employing human-derived primary cells as a target in such investigations.

Funding: The PlasticHeal project has received funding from the European Union's Horizon 2020 research and innovation programme under grant agreement No 965196. This study was supported by the Spanish Ministry of Science and Innovation (PID2020-116789RB-C43) and the Generalitat de Catalunya (2021-SGR-00731).

Fluorescent labeling of micro/nanoplastics for biological applications with a focus on “true-to-life" tracking

Aliro Villacorta[1,2*†], Camila Cazorla-Ares[3†], Victor Fuentes-Cebrian[3], Iris H. Valido[3], Lourdes Vela[1,4], Fernando Carrillo-Navarrete[5], Michelle Morataya-Reyes[1], Karen Mejia-Carmona[6], Susana Pastor[1], Antonia Velázquez[1], Jessica Arribas Arranz[1], Ricard Marcos[1], Montserrat López-Mesas[3], & Alba Hernández[1]

[1] Group of Mutagenesis, Department of Genetics and Microbiology, Faculty of Biosciences, Universitat Autònoma de Barcelona, Cerdanyola del Vallès, 08193, Spain
[2] Facultad de Recursos Naturales Renovables, Universidad Arturo Prat, Iquique, Chile
[3] GTS Research Group, Department of Chemistry, Faculty of Science, Universitat Autònoma de Barcelona, Cerdanyola del Vallès, 08193, Barcelona, Spain
[4] Faculty of Health Sciences Eugenio Espejo, Universidad UTE, Quito, Ecuador
[5] Institut d’Investigació Tèxtil i Cooperació Industrial de Terrassa (INTEXTER) and Department of Chemical Engineering, Universitat Politècnica de Catalunya, Terrassa, 08222, Barcelona, Spain
[6] Institut Català de Nanociència i Nanotecnologia (ICN2-UAB-CSIC-BIST), Cerdanyola del Vallès Spain
* ajvillacorta@gmail.com
† *equal contribution*

The increased environmental presence of micro/nanoplastics (MNPLs), and the potential health risks associated with their exposure, classifies them as environmental pollutants of special environmental/health concern. Consequently, there is an urgent need for studies on the potential risks of secondary MNPLs. In this context, the use of “true-to-life” MNPLs resulting from the laboratory degradation of plastic goods, looks like a sound approach. Nevertheless, these non-commercial secondary MNPLs need to be *labeled* to track their presence/journey inside cells or organisms by using methods such as flow cytometry and confocal microscopy. Since cells are commonly analyzed by fluorescence techniques, and the use of fluorescent dyes seems to be a simple way to stain MNPLs, five different compounds comprising two chemical dyes (Nile Red and Rhodamine-B), one optical brightener (Opticol), and two industrial dyes (Amarillo Luminoso and iDye PolyPink) have been tested to determine their potential for such use. Using commercial standards of polystyrene nanoplastics (PSNPLs) with an average size of 170 nm, different characteristics of the selected dyes such as the absence of cell viability, specificity for plastic staining, no leaching, and lack of interference with other fluorochromes were determined. From the overall data obtained data in wide battery of assays performed, iDye PolyPink was the dye showing more advantages, regarding the other compounds, to be chosen as an effective dye to label “true-to-life” MNPLs. These advantages were confirmed using titanium-doped PETNPLs (obtained from the degradation of milk PET plastic bottles), as an example of true-to-life secondary NPLs. The results confirmed the usefulness of iDye PolyPink to label MNPLs, permitting cell internalization detection.

Funding & Aknowledgment: A. Villacorta was supported by PhD fellowships from the National Agency for Research and Development (ANID), from the CONICYT PFCHA/DOCTORADO BECAS CHILE/2020-72210237. L. Vela was supported by a Ph.D. fellowship from the Fundación Carolina. M. Morataya-Reyes hold a Ph.D. FI fellowship from the Generalitat de Catalunya. A. Hernández was granted an ICREA ACADEMIA award. I.H. Valido was supported by “Ayudas Margarita Salas para la formación de jóvenes doctores”, Ministerio de Universidades (Spain). This project has received funding from the European Union’s Horizon 2020 Research and Innovation Programme under Grant Agreement No. 965196. This work was also partially supported by the Spanish Ministry of Science and Innovation [PID2020-116789, RB-C43], and by the Generalitat de Catalunya (2021-SGR-00731 and 2021-SGR-00723).

Electrochemical sensor for sucralose and its genotoxic ester. A mathematical description

Volodymyr V. Tkach[1,2*], Nataliia M. Storoshchuk[1], Isabel Gaivão[3], Yana G. Ivanushko[4], Petro I. Yagodynets[1], Tetiana V. Morozova[5], José I. Martins[2], & Jarem R. Garcia[6]

[1] Chernivtsi National University, Ukraine
[2] Faculdade de Engenharia da Universidade do Porto, Portugal
[3] Universidade de Trás-os-Montes e Alto Douro, Portugal
[4] Bukovinian State Medical University, Ukraine
[5] National Transport University, Ukraine
[6] Universidade Estadual de Ponta Grossa, Brasil
* nightwatcher2401@gmail.com

Sucralose is one of the most used sweeteners in Portugal and throughout the European Union, in the food and pharmaceutical industries, as a flavor corrector under registration number E955. It is a trichlorosubstituted derivative of galactosucrose, and has twice the sweetness of saccharin, triple the sweetness of aspartame and is up to a thousand times sweeter than common sugar. Sucralose is synthesized from sucrose by a three-stage process. Although sucralose is considered safe for use by diabetics and athletes, its harmful effects on human health and the environment are still little explored, and some of its negative effects have only begun to be studied now. Furthermore, it is necessary to consider the presence of its genotoxic precursor, 6-acetylsucralose, in industrial samples and even in foods. For this reason, the electrochemical determination of 6-acetylsucralose in the presence of sucralose is an option to be considered, and, although the anodic process is also viable, the cathodic process would be more effective from an electroanalytical point of view. The electroanalytical and electrocatalytical process, used to remove both chloroorganic compounds, which are sucralose and its 6-acetyl derivative, may be realized on the graphene electrode, modified by the polymer of mycotoxin necatorin from the *L. Necator* mushroom. This process becomes interesting from the point of view both genotoxicology and circular economy. In this work the electrochemical process for surcalose and its ester on poly(necatorin)-modified cathode is theoretically evaluated. Analysis of the behavior of the system, described by the set of differential equations confirms the effectiveness of this electrochemical process for both the detection of compounds and their removal.

Acknowledgements: Volodymyr V. Tkach thanks the Faculty of Engineering of the University of Porto and the University of Trás-os-Montes and Alto Douro for their support in difficult times for Ukraine and its science.

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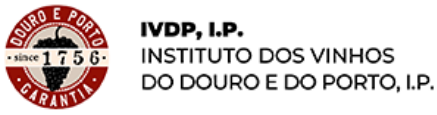
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ABSTRACT BOOK

June 26th - 28th, 2024
Vila Real (Portugal)

SEMA | 2024

28th Spanish Environmental Mutagenesis and Genomics Society (**SEMA**) meeting

https://ojs.diffundit.com/index.php/sema/issue/view/93

sema
Sociedad Española de Mutagénesis y Genómica Ambiental