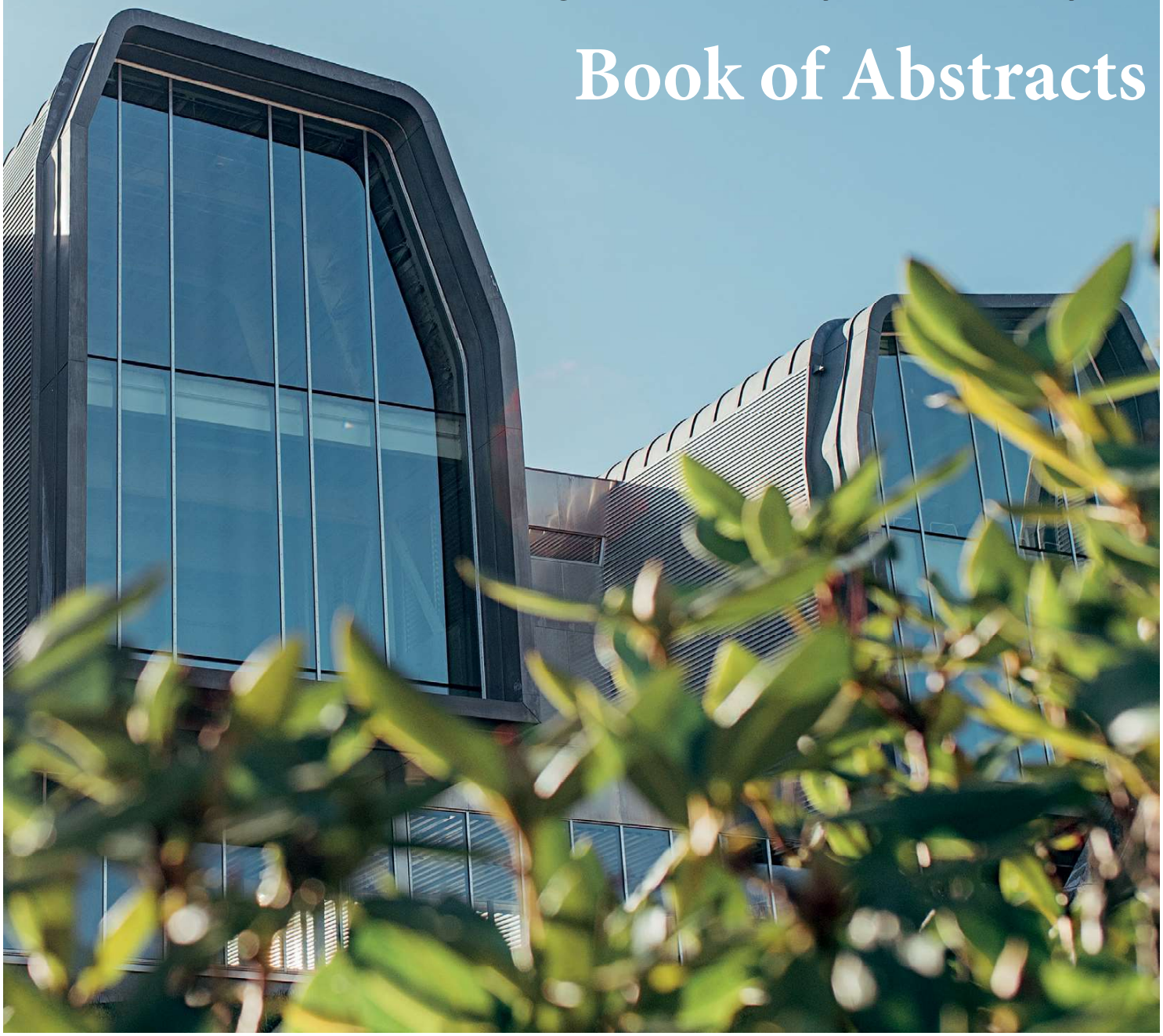
1st & 2nd of July

Faculty of Biology, Vigo, Spain

3 Annual Meeting

From bench to bedside: diagnosis, therapy & data analysis

Book of Abstracts



Universidade Vigo

Centro de Investigacións Biomedicas

Facultade de Bioloxía

Unión Europea

XUNTA DE GALICIA

galicia

DEPUTACIÓN PONTEVEDRA

celta INGENIEROS

fisher scientific part of Thermo Fisher Scientific

Portomédica APARATOS Y MATERIAL MÉDICO HOSPITALARIO

PQN PROQUINORTE

3rd Annual Meeting Cinbio
Biomedical Research Centre
University of Vigo

Book of Abstracts
First edition 2019

Created by:
Organizing & Scientific Committee

I.S.B.N.: 978-84-17934-14-9

Organizing & Scientific Committee

Dr. Miguel Arenas (S)(O)
(XB5) Evolutionary and Biomedical Genomics

Dr. Loretta De Chiara (S)(O)
(BB1) Molecular Biomarkers

Dr. Marta Cousido-Rocha (S)(O)
(IO1) Biostatistics and Epidemiology

José María Esteban (O)
Biomedical Research Center

Jorge Giraldez (O)
(CI8) Analytical & Food Chemistry. Biotoxins

Dr. Sergio Gómez-Graña (S)(O)
(QF1) Colloid Chemistry

Chus Longa Sayáns (O)
(IO1) Biostatistics and Epidemiology

Dr. Hugo López-Fernández (S)(O)
(SI4) Next Generation Computer Systems Group

Dr. Carmen P. Gómez (S)(O)
(FA2) Applied Physics Group

Dr. Sonia Prado-López (S)(O)
(XB5) Evolutionary and Biomedical Genomics

Dr. Rosana Simón-Vázquez (S)(O)
(IN1) Immunology

Dr. Ana Sousa-Castillo (S)(O)
(TNT) Team Nanotech

Dr. José A. Souto (S)(O)
(QO1) Organic Chemistry

Dr. María Dolores Torres (S)(O)
(EQ2) Biomass and Sustainable Development

Dr. Lorena Vázquez-Iglesias (S)(O)
(QF1) Colloid Chemistry

(O) Organizing Committee (S) Scientific Committee

Sponsors

Promover o desenvolvemento tecnolóxico, a innovación e unha investigación de calidade.
FEDER. Unha maneira de facer Europa. Centro Singular de investigación de Galicia 2016-2019

Promover o desenvolvemento tecnolóxico, a innovación e unha investigación de calidade.
FEDER. Unha maneira de facer Europa. Centro Singular de investigación de Galicia 2016-2019

Programme

Promover o desenvolvemento tecnolóxico, a innovación e unha investigación de calidade.
FEDER. Unha maneira de facer Europa. Centro Singular de investigación de Galicia 2016-2019

Conference Programme. DAY 1 - 1st July, 2019

FROM BENCH TO BEDSIDE

9:00 - 9:30	**Registration**
9:30 - 10:00	**Opening and Welcome**
Session 1	**Chairs: Dr. Hugo López-Fernández Dr. Carmen P. Gómez**
10:00 - 10:45	**KS1 - Fátima Al-Shahrour.** Spanish National Cancer Research Center (CNIO), Madrid. ***Identifying druggable genetic dependencies for personalized cancer therapy***
10:45 - 11:00	**ST13 - Manuel Mendoza.** Universidade de Vigo, Vigo. ***RADAR: R package for RNA-Seq experiment design and analysis***
11:00 - 11:15	**ST14 - Cláudia Lopes.** International Iberian Nanotechnology Laboratory, INL, Braga. ***Validation of a microfluidic device for the isolation and characterization of circulating tumor cells towards cancer progression monitoring in metastatic breast cancer***
11:15 - 11:30	**ST1 - Alba Nogueira-Rodríguez.** SING Group, Universidade de Vigo. ***Real time Computer-Aided Diagnosis in colorectal cancer using Deep Learning techniques***
11:30 - 12:15	**Coffee & Posters**
Session 2	**Chairs: Dr. Loretta De Chiara Dr. Rosana Simón-Vázquez**
12:15- 12:45	**IS2 - Angel Díaz-Lagares.** IDIS, CIBERONC, Oncomet, Santiago de Compostela. ***Epigenetic biomarkers in cancer: moving towards liquid biopsy***
12:45 - 13:15	**IS3 - Carmen Álvarez-Lorenzo.** IDIS, Pharmacy, USC, Santiago de Compostela. ***Medical devices as drug delivery platforms: nanotechnology aids and translational issues***
13:15 - 13:30	**ST2 - Olivia Estévez Martínez.** CINBIO-Universidade de Vigo. ***Differential gene expression analysis for the identification of different TB infection profiles***
13:30 - 13:45	**ST3 - Saida Ortolano.** IIS-Galicia Sur- H. Álvaro Cunqueiro, Vigo. ***Functional evaluation of an AAV9 vector expressing alpha-Galactosidase A for potential gene therapy of Fabry disease***
13:45 - 15:30	**Lunch & Networking**
Session 3	**Chairs: Dr. Sergio Gómez-Graña Dr. Ana Sousa-Castillo**
15:30 - 16:00	**IS4 - Alejandro Baeza García.** ETSI Aeronautics and Space, UPM, Madrid. ***Targeting 2.0. Towards a rational design to selective nanocarriers in oncology***
16:00 - 16:15	**ST4 - Marcelina Abal Sanisidro.** Instituto de Investigación Sanitaria, IDIS, Santiago de Compostela. ***Development and evaluation of nanosystems for Photodynamic Therapy triggered by bioluminescence***
16:15 - 16:30	**ST5 - Juan L. Paris.** International Iberian Nanotechnology Laboratory, INL, Braga. ***Fluorescence Cross-Correlation Spectroscopy as a tool to guide the formulation of liposome-coated polycation-DNA complexes (lipopolyplexes) for gene therapy***
16:30 - 17:00	**Coffee & Posters**
Session 4	**Chairs: Dr. Marta Cousido-Rocha Dr. Lorena Vázquez-Iglesias**
17:00 - 17:30	**IS5 - María Xosé Rodríguez-Álvarez.** Basque Center for Applied Mathematics (BCAM), Bilbao. ***On the statistical evaluation of diagnostic and prognostic tests: When patient information beyond test's result can make a difference***
17:30-17:45	**ST6 - Marcos Loureiro-Ga.** Instituto de Investigación Sanitaria Galicia Sur, Vigo. ***Optimizing device selection using numerical simulation in Transcathter Aortic Vlave Implantation***
17:45-18:00	**ST7 - Carla Moreira.** CMAT- School Of Sciences - University of Minho, Braga. ***Dealing with measured confounders in observational studies: The impact of type of birth in childhood obesity***

Conference Programme. DAY 2 - 2nd July, 2019

FROM BENCH TO BEDSIDE

Session 1	Chairs: Dr. Sergio Gómez-Graña Dr. José A. Souto
9:30 - 10:15	**KS2 - Jesús M. de la Fuente.** ICMA, Zaragoza. ***Studying the heat effect of nanoparticles***
10:15 - 10:45	**IS6 - Nuria Martínez Sáez.** Utrecht University, Utrecht and Glycotechnology Laboratory, CIC biomaGUNE, San Sebastián. ***Carbohydrate-based vaccine development***
10:45 - 11:00	**ST8 - Alexandra Teixeira.** International Iberian Nanotechnology Laboratory (INL), Braga. ***Development of an ultrasensitive and low cost paper-based SERS sensor for cell differentiation***
11:00 - 11:15	**ST9 - Andrea Mariño López.** Universidade de Vigo. ***Keeping an eye on the eye through plasmonics: Contact lenses with Au/SiO2 m-capsules***
11:15 - 11:30	**ST10 - Paula Rivas Ramirez.** CNRS - Sorbonne Université, Paris. ***Role of TRPC and GLUD channels in mGLU1/5-gated current in dopamine neurons***
11:30 - 12:00	**Coffee & Posters**
Session 2	**Chairs: Dr. Sonia Prado-López Dr. Miguel Arenas**
12:00 - 12:30	**IS7 - Roberto Piñeiro Cid.** Roche-CHUS Joint Unit, Santiago de Compostela. ***Study of the contribution of clusters of CTCs to the metastasis of breast cancer***
12:30-12:45	**ST11 - Inês Mesquita.** Life&Health Sciences Research Institute ICVS, School of Medicine, Braga. ***HIF-1a deficiency dysregulates lipid metabolism and increases susceptibility to Leishmania donovani infection***
12:45-13:00	**ST12 - Rocío Castro Viñuelas.** INIBIC-Complejo Hospitalario Universitario A Coruña (CHUAC). ***Human induced pluripotent STEM cell-lines (iPSc) generation as an in vitro model of hand ostheoarthritis***
13:00 - 13:30	**IS8 - Carla Oliveira.** I3S, University of Porto, Porto. ***Molecular determinants of gastric cancer: predisposing factors and intra-tumoral heterogeneity***
13:30-14:00	**Prizes and closing remarks**

Promover o desenvolvemento tecnolóxico, a innovación e unha investigación de calidade.
FEDER. Unha maneira de facer Europa. Centro Singular de investigación de Galicia 2016-2019

Keynote Speakers

Promover o desenvolvemento tecnolóxico, a innovación e unha investigación de calidade.
FEDER. Unha maneira de facer Europa. Centro Singular de investigación de Galicia 2016-2019

Keynote Speaker (KS1)

Identifying druggable genetic dependencies for personalized cancer therapy

Fátima Al-Shahrour

Bioinformatics Unit at Spanish National Cancer Research Center (CNIO)
Madrid, Spain

Abstract:

The paradigm of personalized medicine is the identification of the appropriate drug for the right patient, using molecular profiles. In Oncology, it is well established that the anticancer drugs are effective in only a small subset of patients. Moreover, many of the new targeted therapies inhibit specific proteins, and they are only effective in tumors that are genetically altered. Consequently, the success of personalized treatment depends on each individual molecular profile, which a priori can be considered as very heterogeneous.

Here, we present new computational approaches based on the analysis and integration of genomic data (mutations, copy number variations or gene expression levels), with functional data (protein essentiality) and pharmacological data. These methods aim to identify those vulnerable molecular alterations that drive tumor progression and could be druggable based on the patient's molecular profile, and propose an individualized therapeutic strategy to guide clinical decision making for cancer patients.

Promover o desenvolvemento tecnolóxico, a innovación e unha investigación de calidade.
FEDER. Unha maneira de facer Europa. Centro Singular de investigación de Galicia 2016-2019

Keynote Speaker (SK2)

Studying the heat effect of nanoparticles

Jesús M. de la Fuente

Aragón Materials Science Institute (ICMA)
Zaragoza, Spain

Abstract:

In the last decades, inorganic nanoparticles have been steadily gaining more attention from scientists from a wide variety of fields such as material science, engineering, physics or chemistry. The very different properties compared to that of the respective bulk, and thus intriguing characteristics of materials in the nanometre scale, have driven nanoscience to be the centre of many basic and applied research topics. In this talk we describe the synthesis and functionalization of magnetic and gold nanoparticles as therapeutic and diagnosis tools against cancer:
Gold nanoprisms (NPRs) have been functionalized with PEG, glucose, cell penetrating peptides, antibodies and/or fluorescent dyes, aiming to enhance NPRs stability, cellular uptake and imaging capabilities, respectively. Cellular uptake and impact was assayed by a multiparametric investigation on the impact of surface modified NPRs on mice and human primary and transform cell lines. Under NIR illumination, these nanoprobes can cause apoptosis. Moreover, these nanoparticles have also been used for optoacoustic imaging, as well as for tumoral marker detection using a novel type of thermal ELISA nanobiosensor using a thermosensitive support.
Magnetic nanoparticles functionalized with DNA molecules and further hybridizing with different length fluorophore-modified DNA have allowed the accurate determination of temperature spatial mapping induced by the application of an alternating magnetic field. Due to the design of these DNAs, different denaturalization temperatures (melting temperature, Tm) could be achieved. The quantification of the denaturalized DNA, and by interpolation onto a Boltzmann fitting model, it has been possible to calculate the local temperature increments at different distances, corresponding to the length of each modified DNA, from the surface of the nanoparticles.

Promover o desenvolvemento tecnolóxico, a innovación e unha investigación de calidade.
FEDER. Unha maneira de facer Europa. Centro Singular de investigación de Galicia 2016-2019

Invited Speakers

Promover o desenvolvemento tecnolóxico, a innovación e unha investigación de calidade.
FEDER. Unha maneira de facer Europa. Centro Singular de investigación de Galicia 2016-2019

Invited Speaker (IS2)

Epigenetic biomarkers in cancer: moving towards liquid biopsy

Ángel Díaz-Lagares

IDIS, CIBERONC, Oncomet
Santiago de Compostela, Spain

Abstract:

In recent years there have been great advances in the characterization of the epigenome through the global study of epigenetic mechanisms. These epigenetic modifications have a very important function in the regulation of gene expression of cells. However, there are certain situations that produce alterations of these mechanisms leading to the development of different diseases such as cancer. DNA methylation is the best studied epigenetic mechanism, showing great clinical utility as a tumor biomarker. So far, this DNA modification has been mostly studied in tumor samples by means of the analysis of the epigenome or individual genes. However, it is increasing the number of studies that show the clinical usefulness of different epigenetic marks as biomarkers in liquid biopsy, which allows to study in a non-invasive way the circulating tumor material (DNA, RNA, circulating tumor cells or CTCs, exosomes) released by the tumors to the different biological fluids. The study of epigenetic biomarkers in liquid biopsy has important advantages over traditional biopsy, since it can provide relevant information on tumor heterogeneity and allows the study of the dynamic evolution of tumors throughout the disease of patients. DNA methylation in liquid biopsy has been studied mainly by the analysis of individual genes or gene panels. However, recently epigenomic tools have started to analyze the epigenetic mechanisms directly in liquid biopsy. This new approach represents a great advance for the epigenomics of cancer favoring the discovery of new epigenetic biomarkers with clinical utility in oncology.

Promover o desenvolvemento tecnolóxico, a innovación e unha investigación de calidade.
FEDER. Unha maneira de facer Europa. Centro Singular de investigación de Galicia 2016-2019

Invited Speaker (IS3)

Medical devices as drug delivery platforms: nanotechnology aids and translational issues

Carmen Álvarez Lorenzo

IDIS, Pharmacy, USC
Santiago de Compostela, Spain

Abstract:

Medical devices play a physical role in diagnosis, therapy and replacement of body tissues or functions. Drug-eluting medical devices may overcome risks inherent to their use (inflammation, infection) and also offer synergic therapeutic features [1,2]. The medical device can control the release of the drug at the site where it is needed and, consequently, the efficacy and the safety of the treatment, as well as its cost-effectiveness are improved.
Methods for surface modification with stimuli-responsive polymers or with networks that can selectively recognize certain biomarkers or cells have been implemented for feed-back modulated drug release [3]. A wide range of techniques for the grafting of functional nanobrushes is currently available [4].
Nanostructuring of device matrix may render artificial receptors for the drug, exhibiting affinity-regulated mechanisms for the hosting and the release of the drug. Surface functionalization allows for the release on-demand of drugs and biological products, being switchable on/off as a function of the progression of certain physiological or pathological events (e.g., healing, body integration, biofilm formation) [5].
Although in vivo tests are still limited, the knowledge on the drug-device-body interactions and the application of revolutionary nanotechnology approaches may pave the way to the design of drug-eluting medical devices with optimized and novel performances.

Acknowledgements: MINECO [SAF2017-83118-R], AEI Spain, Xunta de Galicia [ED431C 2016/008; ED431E 2018/08], and FEDER.

References:

[1]Concheiro A., Alvarez-Lorenzo C. In: Smart Materials for Drug Delivery. Royal Society of Chemistry, vol. 2, RSC, UK, 2013, pp. 313-348.
[2]Brackman G., et al. Macromol. Biosci. 16 (2016) 859.
[3]Gonzalez-Chomón C., et al. Acta Biomater. 41 (2016) 302.
[4]Cabana S., et al. J. Drug Deliv. Sci. Tech. 42 (2017) 245.
[5]Segura T., et al. Biomacromolecules 15 (2014) 1860.

Invited Speaker (IS4)

Targeting 2.0. Towards a rational design to selective nanocarriers in oncology

Alejandro Baeza García

ETSI Aeronautics and Space, UPM
Madrid, Spain

Abstract:

Current treatment of cancer is usually based on the administration of potent cytotoxic drugs able to destroy dividing cells as tumoral ones. Chemotherapy has constituted the main therapeutic approach for the treatment of aggressive tumors for over 100 years. However, one of its main drawbacks is the lack of selectivity which leads to numerous side effects and compromise the patient life. In the recent years, special attention has been devoted to the design of nanocarriers able to selectively transport these cytotoxic drugs to tumoral cells and once there, to release their cargo in a controlled manner in the presence of certain stimuli. Nanoparticles injected into the blood stream tend to be accumulated in tumoral tissues as consequence of the high permeability of tumoral blood vessels and the lymphatic system collapse, a phenomenon known as Enhanced Permeation and Retention effect (EPR). This property is extremely important in oncology because it supposes that it could be possible to deliver therapeutic drugs to tumoral tissues simply loading them inside nanoparticles. However, the real scenario is more complex. Tumoral tissues are composed by a myriad of different cell populations as immune, supportive and healthy tisular cells, not only tumoral ones. Therefore, if we want to eradicate only cancerous cells, the capacity to distinguish them should be provided to the nanoparticle. The incorporation of targeting abilities on nanocarriers has been traditionally addressed through the grafting of different biomolecules such as antibodies, proteins, vitamins or small peptides. Herein, we are going to describe a slightly different approach which consists in the synthesis ad hoc of small molecules which present strong affinity by certain membrane receptors overexpressed on the surface of tumoral cells. These molecules can be anchored on the surface of different types of nanoparticles driving them directly to the tumoral cells. Additionally, the design of hierarchical targeting strategies to improve the accumulation of nanocarriers in tumoral tissues and then, trigger the endocytosis into the cancerous cell only in the presence of certain stimuli, or to drive the nanocarriers close to certain organelles, will be described.

Promover o desenvolvemento tecnolóxico, a innovación e unha investigación de calidade.
FEDER. Unha maneira de facer Europa. Centro Singular de investigación de Galicia 2016-2019

Invited Speaker (IS5)

On the statistical evaluation of diagnostic and prognostic tests: When patient information beyond test's result can make a difference

María Xosé Rodríguez- Álvarez

Basque Center for Applied Mathematics, BCAM
IKERBAQUE, Basque Foundation for Science
Bilbao, Spain

Abstract:

Accurate diagnosis or prognosis of diseases is of fundamental importance in clinical practice, and diagnostic tests are usually used to aid in the decision. A diagnostic test can be any procedure conducted to differentiate between different types of patients, e.g. healthy versus diseased, or patients in different stages of disease progression. However, the classification of an individual's status based on the result of a test is usually not error-free, and some individuals will be misclassified. Thus, before using a test in a routine clinical setting, any errors of classification must be quantified to check the test's validity or invalidity, i.e. diagnostic accuracy must be measured. However, it is usually overlooked that the accuracy of a test may be affected by external factors. Examples of such factors include different test settings and subject-specific characteristics. For instance, the accuracy of a test may be different in males and females, or it may vary according to the age of the individual. In this talk, we will motivate and discuss the importance of accounting for such external factors when evaluating the performance of a test. Through toy examples and real data, we will show that failure to incorporate such factors in the statistical analysis may lead to erroneous conclusions. Emphasis will be placed on the receiver operating characteristic (ROC) curve, unarguably, the most popular tool for evaluating the accuracy of a test. We will introduce the conditional ROC curve, an extension of the ROC curve which allows including modifying factors. We will show that the conditional ROC curve can be viewed as a tool which helps to identify those patients' strata that may benefit from the application of the test, as well as those for which the test does not provide valuable information. Software allowing the estimation of conditional ROC curves will also be discussed.

Promover o desenvolvemento tecnolóxico, a innovación e unha investigación de calidade.
FEDER. Unha maneira de facer Europa. Centro Singular de investigación de Galicia 2016-2019

Invited Speaker (IS6)

Carbohydrate-based vaccine development

Nuria Martínez Saez

Utrecht University, Utrecht, The Netherlands
Glycotechnology Laboratory, CIC biomaGUNE, San Sebastián, Spain

Abstract:

Pathogenic extracellular bacteria often express high molecular weight Capsular Polysaccharides (CPs) on their surface. These carbohydrates are optimal targets for the development of microbial polysaccharide-based vaccines, and their use in vaccination avoids the administration of potentially harmful microbial components. However, Capsular Polysaccharides are T-cell independent antigens that can be used to prevent infections in adults, nevertheless, in high-risk groups such as neonates or elderly such vaccines elicit poor antibody responses. This limitation can be overcome by conjugation of the CPs to a carrier protein.[1] Fragment HCR of Tetanus neurotoxin (TeNT) protein will be used as a source of T-helper epitopes.
A major challenge for the construction of glycoconjugate vaccines is the development of site-specific techniques to incorporate the oligosaccharides to the protein. Site-specific modifications generate proteins with a precise and defined structure, maintaining their function intact and avoiding any damage of their immunogenic epitopes. This is a significant advantage over conventionally used random conjugation strategies. In this regard, we have developed a new methodology by using N-terminus-specific enzyme-mediated ligation strategies for protein modification.[2] We have used a peptide ligase enzyme (Omniligase), that allows the incorporation of peptides at the N-terminal site of a protein, and these peptides can be equipped with a variety of biorthogonal reactive groups for their subsequent chemical modification with CPs.
As CPs, we have chosen the Streptococcus pneumoniae 35B antigen, since Streptococcus pneumoniae infection still causes the death of around 500,000 children under 5 years of age each year.[3] We have designed an innovative synthetic strategy for the obtaining of capsular antigens with different lengths to determine the length for optimal immunogenicity. The antigens display an alkyne that serves as a substrate for strain promoted azide-alkyne cycloaddition (SPAAC) reaction. The peptides attached by the Omniligase to the protein show either one azido or three azido functional groups. Therefore, these glycoconjugates allow us to explore the impact of the multivalent presentation of the antigens in the immune response.

[1] J. A. Jaurigue, P. H. Seeberger, *Front Cell Infect Microbiol* **2017**, *7*, 248-248.
[2] C. B. Rosen, M. B. Francis, *Nat. Chem. Biol.* **2017**, *13*, 697.
[3] S. S. Richter, D. M. Musher, *J. Clin. Microbiol.* **2017**, *55*, 681-685.

Promover o desenvolvemento tecnolóxico, a innovación e unha investigación de calidade.
FEDER. Unha maneira de facer Europa. Centro Singular de investigación de Galicia 2016-2019

Invited Speaker (IS7)

Study of the contribution of CTC clusters to breast cancer metastasis

Roberto Piñeiro Cid

Roche-CHUS Joint Unit
Santiago de Compostela, Spain

Abstract:

The vast majority of cancer-related deaths are caused by cancer metastasis, process by which tumor cells separate from the primary tumor, travel through the blood as circulating tumor cells (CTCs), and form new tumors in distant tissues or organs. CTCs are found in the blood of cancer patients as single cells, although small groups of these cells named CTC clusters have also been detected. CTC clusters seem to have greater metastatic potential than individual CTCs, and their presence in the blood of breast cancer patients is associated with poorer prognosis. However, the actual contribution of CTC clusters to metastasis is not well understood and further studies are needed. Our work aims to study the biology of CTC clusters through their functional and molecular characterization by using preclinical models, in order to understand their role in metastasis. Our results show how CTC clusters have differential properties compared to individual CTCs, which could contribute to their higher metastatic potential.

Promover o desenvolvemento tecnolóxico, a innovación e unha investigación de calidade.
FEDER. Unha maneira de facer Europa. Centro Singular de investigación de Galicia 2016-2019

Invited Speaker (IS8)

Molecular determinants of gastric cancer: predisposing factors and intra-tumoral heterogeneity

Carla Oliveira

Institute of Investigation and Innovation in Health (I3S) and Institute of Molecular Pathology and Immunology, University of Porto (IPATIMUP)
University of Porto, Portugal

Abstract:
Gastric cancer is the third leading cause of cancer-related deaths worldwide, affecting close to one million people per year. Over 90% of all gastric cancers appear in a sporadic setting, less than 10% show familial clustering, and of these, only 1% to 3% constitute hereditary forms.
The majority of gastric cancer patients present with advanced disease at diagnosis, rendering the prognosis extremely poor, with a 5-year overall survival rate of less than 25%. Treatment of GC patients is often not efficient, as all patients are treated similarly regardless of the disease subtype. Next-generation sequencing data is increasing the knowledge of the molecular basis of gastric cancer and the comprehensive study of genomic and epigenomic alterations associated with this disease. Nonetheless, the clinical usefulness of these discoveries is still limited due to the lack of systematic genetic-clinical correlations for personalized use.
We have studied the molecular heterogeneity of HER2 positive sporadic gastric cancers by whole genome sequencing and consecutive immunohistochemistry with image registration and overlay and determined novel therapy targets in these cancers. We have also demonstrated that anti-HER2 based therapy can be improved with photodynamic therapy.
In the hereditary setting of GC, and particularly in Hereditary diffuse gastric cancer (HDGC), we performed the first genotype-phenotype correlation analysis in HDGC families harbouring germline causative variants in CDH1. We also identified novel germline defects likely explaining 6% of the families and proved that monoallelic CDH1 downregulation is present in the germline of most HDGC-families that remain mutation negative. To identify the genetic mechanism underlying monoallelic CDH1 downregulation, we sequenced (NGS) the full CDH1 locus of >300 bonafide HDGC CDH1-negative families. Noncoding variants (NCVs) were bioinformatically prioritized according to their absence from public genome databases and rareness within this cohort. NCVs were integrated with regulatory functional annotation to select iCREs, whose function was evaluated through in vitro and in vivo (zebrafish and mouse) reporter assays and CRISPR-Cas9-mediated iCRE deletion.
A set of potential iCREs was defined within CDH1, and some of them were shown to act as enhancers in vitro and in vivo. CRISPR/Cas9 edition of iCREs in cell lines led to massive CDH1 mRNA downregulation and E-cadherin loss of expression; while heterozygous deleted clones showed a strong monoallelic downregulation, recapitulating the phenotype observed in HDGC patients. These results support our assumption that identified iCREs are true regulatory elements modulating CDH1/E-cadherin expression. High numbers of clinically homogeneous HDGC families and a locus-targeted approach allowed identifying new CDH1 inactivating mechanisms likely causative in HDGC.
Funding: Portuguese FCT Grant Ref. PTDC/BTM-TEC/30164/2017; American NSFC Foundation - Grant Ref. NSFC_2014, EU-Horizon 2020/SolveRD Project Grant Ref. 779257; Ipatimup Board of Directors; European Reference Network GENTURIS

Oral Communications

Promover o desenvolvemento tecnolóxico, a innovación e unha investigación de calidade.
FEDER. Unha maneira de facer Europa. Centro Singular de investigación de Galicia 2016-2019

Oral Communication (ST1)

Real time computer-aided diagnosis in colorectal cancer using Deep Learning techniques

Nogueira-Rodríguez A[1,2], López-Fernández H[1,2], Iglesias Gómez A[3], Herrero Rivas JM[3] , Rivas Moral L[3], Puga Giménez de Azcarate M[3], Sánchez Hernández E[3], Remedios Espino D[3], Fdez-Riverola F[1,2], Cubiella Fernández J[3], Reboiro-Jato M[1,2], Glez-Peña D[1,2]

1. Department of Computer Science, University of Vigo, ESEI, Spain. 2. The Biomedical Research Centre (CINBIO), Vigo, Spain. 3. Complejo Hospitalario Universitario de Ourense (CHUO), Sistema Galego de Saúde (SERGAS), Ourense, Spain.

Abstract:

Colorectal cancer is one of the most prevalent types of cancer in Spain (34,331 new patients in 2017 and 15,802 deaths in 2016). Its development starts with the appearance of polyps or neoplastic lesions in the colon which may evolve into malignant tumors. If a polyp is detected during endoscopy, a resection is carried out in order to perform a biopsy of the extracted tissue. Nevertheless, it may happen that some resections are not really necessary, thus performing an unnecessary and avoidable analysis. In the last decades, there has been a development of different optical technologies to help endoscopists in the identification of the histological diagnosis of the polyps. Since these techniques require highly experienced practitioners, there has been also a lot of interest in the development of Computer-Aided Diagnosis systems able to predict the histology in real-time during endoscopy, avoiding unnecessary polyp resections and biopsies. Recently, Deep Learning, an Artificial Intelligence technique, has become an important player in the field of medical image analysis due to its higher performance in image classification tasks when compared to previous state-of-the-art techniques. In this context, this contribution presents an ongoing project aiming to develop a real-time colon polyp detection, localization and classification system based on Deep Learning techniques. At the core of the system there will be three models: (i) the polyp detection model, which will be used to predict whether a given image contains a polyp or not; (ii) the polyp localization module, which will be used to take positive images from the detection model and predict the location of the lesion; and (3) the polyp classification model, which will be used to predict the histological diagnosis. It is expected that the creation of this system will help endoscopists in the optical diagnosis of colon lesions, giving an observer-independent aid during colonoscopy.

Promover o desenvolvemento tecnolóxico, a innovación e unha investigación de calidade.
FEDER. Unha maneira de facer Europa. Centro Singular de investigación de Galicia 2016-2019

Oral Communication (ST2)

Differential gene expression analysis for the identification of different TB infection profiles

Estevez O[1,2], Anibarro L[2,3], Garet E[1,2], Pallares A[4], Barcia L[3], Pena A[3], Maueia C[5], Mussá T[5,6], Fdez-Riverola F[1,2,7], Glez-Peña D[1,2,7], Reboiro-Jato M[1,2,7], López-Fernández H[1,2,7], Fonseca N[8,9], Reljic R[10], González-Fernández A[1,2]

1. Centro de Investigaciones Biomédicas (CINBIO), University of Vigo, Spain. 2. Galicia Sur Health Research Institute (IIS Galicia Sur), SERGAS-UVIGO. 3. Tuberculosis Unit, Department of Infectious Diseases and Internal Medicine, Pontevedra Hospital Complex, Pontevedra, Spain. 4. Department of Microbiology, Pontevedra Hospital Complex, Pontevedra, Spain. 5. Departamento de Plataformas Tecnológicas, Instituto Nacional de Saúde. Ministério da Saúde, Moçambique. 6. Department of Microbiology, Faculty of Medicine, Eduardo Mondlane University, Maputo, Mozambique. 7. ESEI - Escuela Superior de Ingeniería Informática, University of Vigo, Ourense, Spain 8. European Bioinformatics Institute, Cambridge, United Kingdom. 9. CIBIO/InBIO - Research Center in Biodiversity and Genetic Resources, Universidade do Porto, Portugal. 10. St. George's, University of London, London, United Kingdom.

Abstract:

One of the current goals in the fight against Tuberculosis (TB) infection is to develop new diagnostic tools that allow a rapid and accurate detection of latent and active TB and the identification of patients with latent TB infection (LTBI) with higher risk of developing active TB.
To find a differential gene expression signature for the different stages of TB infection and identify the LTBI patients suspected of being at higher risk of developing active TB by means of RNAseq analysis.
28 active TB patients and 68 contacts (27 LTBI and 41 uninfected) were enrolled. Participants' whole blood mRNA was isolated and sequenced and the differential expression between groups was calculated using the R package DESeq2. Differentially expressed (DE) genes were used to identify a gene signature that differentiates active TB from both contact groups. A machine learning approach was used to create a classification model that allowed the classification of LTBI contacts in two subgroups.
The DE signature that differentiates active TB patients from their contacts include genes involved in the complement cascade, antigen presentation, neutrophil degranulation, production of antimicrobial peptides and re-organization of the extracellular matrix. Additionally, genes coding for immunoglobulin chains differentiate active TB from uninfected contacts. Two subgroups of LTBI contacts were identified. A small percentage (22%) presented an infection-like profile suggestive of a higher risk of developing active TB. These patients were characterized by a higher expression of genes involved in the complement cascade, cytokine signalling and immunoglobulin production.
A gene signature was identified that could be used to differentiate active TB patients and latent infection. This gene signature could help on identifying those LTBI patients at higher risk of developing active TB.

Promover o desenvolvemento tecnolóxico, a innovación e unha investigación de calidade.
FEDER. Unha maneira de facer Europa. Centro Singular de investigación de Galicia 2016-2019

Oral Communication (ST3)

Functional evaluation of an AAV9 vector expressing alpha-Galactosidase A for potential gene therapy of Fabry disease

Ortolano S[1], Biferi MG[2], San Millán B[1], Souto O[3], Vieitez I[1], Cohen M[1], Teijeira S[1], Fernández-Lorenzo JR[1], González-Fernandez A[3], Barkats M[2]

1.IIS-Galicia Sur-Grupo EERR y Med. Pediátrica, Vigo, Spain. 2. Institute de Myologie UMRS974 INSERM/UPMC/AIM, Paris 3. CINBIO, University of Vigo, Vigo, Spain.

Abstract:

Fabry disease (FD) is treated by enzyme replacement therapy (ERT) or pharmacological chaperons (in UE). However, ERT has a short half-life and cannot cross the blood brain barrier (BBB), while pharmacological chaperons are not indicated for all diagnosed patients. We aim to test the efficacy of a gene therapy vector for FD, based on self complementary Adeno-Associated-Virus9 (scAAV9). The vector (AAV9_PGK_GLA) expresses human α-GalactosidaseA (rh-α-GalA) under the promoter phosphoglycerate-kinase1. This vector can potentially achieve systemic expression of rh-α-GalA and cross the BBB. AAV9_PGK_GLA was injected in hemizygous B6;129-Glatm1Kul/J newborns (P1, n=12) and the animals were sacrificed 3 or 5 months post-injection. rh-α-GalA activity was measured in brain, spinal cord, liver, heart and kidney and glycosphynsolipid deposits were detected by immunohistochemistry. rh-α-GalA is functional in all the analyzed tissues including the CNS and remains stable after 5 months. Gb3 deposit are less abundant in treated animals compared with not injected knockouts. Afterwards the vector was tested in presymptomatic and symptomatic adults animals at two different dosis and in both hemizygous and heterozygous mice. The protein is functional in all tissues 5 months following injection and Lyso-Gb3 deposit are dramatically decreased in plasma (LC-MS) and in tissues (inmunofluorescence). IgG antibodies against t rh-α-GalA were detected in 6 out of 32 injected animals, however the enzymatic activity in tissues is not affected.

Promover o desenvolvemento tecnolóxico, a innovación e unha investigación de calidade.
FEDER. Unha maneira de facer Europa. Centro Singular de investigación de Galicia 2016-2019

Oral Communication (ST4)

Development and evaluation of nanosystems for Photodynamic Therapy Triggered by bioluminescence

Abal-Sanisidro M[1], Díez-Villares S[1], Alijas S[1], Carreira-Rodríguez R[2], G Blanco M[2], de la Fuente M[1]

1. Nano-oncology Unit, Health Research Institute of Santiago de Compostela (IDIS), Santiago de Compostela, Spain. 2. DNA repair & genome integrity lab, Center for Research in Molecular Medicine and Chronic Diseases (CiMUS), Santiago de Compostela, Spain.

Abstract:

Photodynamic Therapy (PDT) has proven to be very efficient for treating external lesions such as melanoma. However, due to the poor penetration of light, PDT it is not suitable for treating internal organs, like pancreas. For this particular purpose, we aim to develop self-illuminating nanoparticles, and prove the potential on an innovative therapeutic nanomedicine activated by bioluminescence to mediate a localized and controlled antitumoral response in pancreatic cancer.
Self-illuminating nanoparticles were successfully developed by the conjugation of a mutant of the Renilla Luciferase (RLuc8) to quantum dots (QDots). A pBAD-RLuc8 plasmid (kindly supplied by Dr. Gambhir in Stanford University) was successfully transformed into LMG194 E. Coli strain and purified. The functionality of RLuc8 was determined by measuring its bioluminescence intensity after adding a substrate (coelenterazine) showing a great signal. The conjugation of the protein to the quantum dots (RLuc8-QDots) was performed by EDC coupling forming an amide group between the primary amine group of the protein and the carboxylic groups set on the surface of the QDots. The success of the conjugation was proved by performing electrophoresis gels analysis, and by determining the BRET ratio after the addition of the substrate. With respect to their interaction with pancreatic cancer cells, confocal microscopy revealed an efficient internalization of the conjugates, and the potential of the developed technology. In vitro studies are currently being performed in order to evidence the formation of Reactive Oxygen Species (ROS) heading cells to death.
In conclusion, we have developed and characterized self-illuminating nanoparticles that are efficiently internalized into cancer cells. Because of this and considering the overlapping wavelengths of the conjugates' components, by BRET, this nanosystem activates a photosensitizer, proving that these nanoparticles can provoke ROS and lead cancer cells to apoptosis.

Promover o desenvolvemento tecnolóxico, a innovación e unha investigación de calidade.
FEDER. Unha maneira de facer Europa. Centro Singular de investigación de Galicia 2016-2019

Oral Communication (ST5)

Fluorescence Cross-Correlation Spectroscopy as a tool to guide the formulation of liposome-coated polycation-DNA complexes (lipopolyplexes) for gene therapy

Paris JL, Gaspar R, Coelho F, De Beule P, Silva BFB

International Iberian Nanotechnology Laboratory, Braga, Portugal

Abstract:

Gene delivery vectors hold great promise for the development of therapeutics. While viral vectors can provide successful gene transfection in vivo, safety concerns associated with them make non-viral vectors a more appealing alternative for future therapies. However, the in vivo transfection efficiency of non-viral vectors has been very limited so far, and part of this issue is due to an incomplete understanding of the optimal parameters needed in these nano-formulations. A clear example of this issue are complexes of polycations, lipids and nucleic acids (usually called lipopolyplexes). While lipopolyplexes have been widely proposed as useful nanotherapeutics, to the best of our knowledge, no work has yet evaluated in a quantitative manner the degree of success in obtaining these three-component structures.
In this work, we have prepared a library of different lipid-polylysine-DNA strucures, characterizing them by Dynamic Light Scattering (DLS), Z Potential, Transmission Electron Microscopy (TEM) and Fluorescence Cross-Correlation Spectroscopy (FCCS). FCCS allows the evaluation particle diffusion in two spectrally-separated channels, revealing the presence or absence of interactions between the differently-labeled components. Employing Atto 488-labeled polylysine and Texas Red-labeled lipids, we have shown for the first time that FCCS allows us to evaluate the degree of co-localization of the polymeric and lipidic components in a quantitative manner. This degree of cross-correlation was found to vary greatly with different ratios of the main components, as well as with the presence or absence of a polyethylene glycol (PEG)-grafted lipid, which is expected to have significant implications for their therapeutic use. The results here obtained show that FCCS can become a powerful tool in the context of nanoformulation design, providing new insights that cannot be achieved by traditional characterization techniques.

Promover o desenvolvemento tecnolóxico, a innovación e unha investigación de calidade.
FEDER. Unha maneira de facer Europa. Centro Singular de investigación de Galicia 2016-2019

Oral Communication (ST6)

Optimizing device selection using numerical simulation in Transcathter Aortic Vlave Implantation

Loureiro-Ga M[1,2], Veiga C[1], Fdez-Manin G[2], Salvadores PJ[1], Jimenez Diaz VA[1], Baz JA[1], Sonntag SJ[3], Iñiguez A[1]

1. Servicio de Cardioloxía, Hospital Álvaro Cunqueiro - Instituto de Investigación Sanitaria Galicia Sur, SERGAS-UVIGO, Vigo, Spain. 2. Departamento de Matemática Aplicada II - EE Telecomunicacións, Universidade de Vigo, Vigo, Spain. 3. Virtonomy.io, Munich, Germany

Abstract:

Transcatheter Aortic Valve Implantation (TAVI) allows the delivery of an artificial valve in the aortic position using a catheter inserted from the peripheral vessels along the patient's arteries instead of an open-heart operation. Once a patient is proposed to undergo a TAVI intervention, some factors such as the percutaneous access, the device size or the device model, among others, need to be considered. Also during the intervention, the proper radial tension needs to be administered in order to ensure a proper anchoring to the aortic annulus.

The main purpose is to assess some risk factors and possible intervention outcomes in advance. Some of those factors can be the election of the right size of the device or to check the behavior of different valves for the same patient.

The first step is to create a 3D- personalized model of the geometry of the patient under study. The images from the CT scan which is done in advance are processed in such a way that a 3D reconstruction is obtained.

Our research team has designed different sizes of the Edwards Sapien TAVI device. Those devices are virtually deployed inside of the geometry of each patient using solid mechanics numerical simulation analysis with the finite elements method (FEM).

The process was successfully done for a patient to whom a 23mm and a 26mm Edwards Sapien XT TAVIs were virtually implanted. The simulations showed that the 26mm size valve anchored better producing a better sealing.

This work shows the potential use of numerical simulation techniques in the clinical practice when planning the interventions, showing some additional and valuable information.

Oral Communication (ST7)

Dealing with measured confounders in observational studies: The impact of type of birth in childhood obesity

Moreira, C[1], Camblor-Martinez P[3], Cristina Santos A[2.4], Barros H[2.4]

1. CMAT- School of Sciences, University of Minho, Braga, Portugal. 2. EPIUnit - Instituto de Saúde Pública, Universidade do Porto, Porto, Portugal. 3. The Dartmouth Institute for Health Policy and Clinical Practice, Geisel School of Medicine, Dartmouth College, Hanover (NH), USA. 4. Departamento de Ciências da Saúde Pública e Forenses e Educação Médica, Faculdade de Medicina, Universidade do Porto, Porto, Portugal.

Abstract:

Childhood obesity has been linked to cesarean section (CS) via lack of exposure to vaginal microflora although the literature is inconsistent. We investigated the association between type of delivery and the risk of childhood obesity among singletons without congenital abnormalities of the birth cohort Generation XXI, at the 4th, 7th and 10th years old of follow-up. We compare four different methods to control for potential measured confounders: multivariate logistic regression, propensity score-matched analysis, propensity score-based weighted method using two different approaches to calculate the propensity scores - via logistic regression model and with a generalized boosted model. The crude odds ratio between CS delivery and obesity/overweight varied from 1.12 (95% confidence interval: 1.01 to 1.27) at 4 years old follow-up to 1.17 (95% confidence interval: 1.01 to 1.27) at 10 years old follow-up. The adjusted odds ratio depended on the adjustment method used, ranging from 1.08 (95% confidence interval: 0.99 to 1.19) for the inverse-probability-of-treatment-weighted analysis with boosted model to 1.25 (95% confidence interval: 1.15 to 1.37) for the inverse-probability-of-treatment-weighted analysis with logistic regression. High levels of nonuniform exposition effect render summary estimates sensitive to the weighting system explicit or implicit in an adjustment technique. The results in this analysis provided similar results, showing that even controlling for potential measured confounders the CS increased the risk of childhood obesity. Althought the observed results are consistente in order to prove the effect of the caesarean on the childhood obesity, it is important to point out the potential effect of unmeasured confounders for which no propensity score method is adjusting for.

Promover o desenvolvemento tecnolóxico, a innovación e unha investigación de calidade.
FEDER. Unha maneira de facer Europa. Centro Singular de investigación de Galicia 2016-2019

Oral Communication (ST8)

Development of an ultrasensitive and low cost paper-based SERS sensor for cell differentiation

Teixeira A*[1], Hernández-Rodríguez JF[2], Wu L[1], Oliveira K[1,3], Kant K[1], Piairo P[1,4], Diéguez L[1], Abalde-Cela S*[1]

1. International Iberian Nanotechnology Laboratory (INL), Braga, Portugal, 2. Department of Analytical Chemistry, Physical Chemistry and Chemical Engineering, University of Alcalá (UAH), Spain. 3. Universidade Nova de Lisboa, Portugal. 4. Instituto de Medicina Molecular, Faculdade de Medicina, Universidade de Lisboa, Lisboa, Portugal.
*Correspondence: alexandra.teixeira@inl.int ; sara.abalde@inl.int

Abstract:

Nanotechnology, surface-enhanced Raman scattering (SERS) spectroscopy and microfluidics are key enabling technologies (KETs) that have been prospering over the last years [1]. SERS is an ultrasensitive sensing technique, that overcomes the intrinsic low efficiency of Raman by using nanoparticles [1,2]. The high sensitivity of this analytical tool allows it to be used in chemical and biological analysis [3]. However, one of the main challenges of the field has been the development of simple, ready-to-use and low-cost SERS substrates that foster the transfer of this technology from the bench to the bedside. Here, we have developed a portable, low-cost and scalable sensor based on SERS for the rapid identification of cell profiles.
First, to control the fabrication of the SERS substrates, the intrinsic reproducibility of microfluidics technology was used for the fabrication of self-assembled nanoparticle structures over a paper film [4]. The paper substrates were fabricated by assembling anisotropic particles, gold nanostars (GNSs) and nanorods (NRs) onto paper to offer an extra enhancement to reach ultrasensitive detection limits. Thus, GNSs and NRs were synthesised by following conventional protocols with few modifications [5,6]. A polymer-paper hybrid device was used for the self-assembly of nanoparticles, which provided the ideal environment to control the drying kinetics of nanoparticles over the paper substrate. This method allowed a high reproducibility and homogeneity of the fabrication of SERS substrates that reached limits of detection down to the picomolar range and this method is quite simple and fast, takes less than 30 minutes and allows the preparation of several substrates simultaneously. A proof-of-concept experiment for the differentiation of two different cell lineages was designed to demonstrate the potential of this SERS on paper substrates for their application in diagnosis.

Promover o desenvolvemento tecnolóxico, a innovación e unha investigación de calidade.
FEDER. Unha maneira de facer Europa. Centro Singular de investigación de Galicia 2016-2019

Oral Communication (ST9)

Keeping an eye on the eye through plasmonics: Contact lenses with Au/SiO2 m-capsules

Mariño-López A, Sousa-Castillo A, Correa-Duarte MA

Department of Physical Chemistry, Center for Biomedical Research (CINBIO), Southern Galicia Institute of Health Research (IISGS), and Biomedical Research Networking Center for Mental Health (CIBERSAM) Universidade de Vigo, Vigo, Spain

Abstract:

Eyes are exposed to a wide range of electromagnetic radiations and some of them, as UV and IR light, can be harmful to them. Several studies have focused their attention in the development of UV-blocking contact lenses, but few researches tackle the topic of IR protection. A prolonged exposure to this type of radiation, coming from the sun or artificially generated, such as lasers, can cause eye damage.

In order to minimize human eye exposure to IR radiation, hydrogel-based soft contact lenses containing gold-silica mesoporous nanocapsules are synthetized. Gold nanoparticles are grown inside the silica capsule and these plasmon resonant structures extinguish large part of near-infrared energy. The electromagnetic shielding capabilities of the hybrid contact lenses were tested. With this aim, high powered lasers and conventional laser pointers operating in the visible and NIR regions were impinged onto the surfaces of the contact lenses and it can be observed a remarkable decrease of the output power of the incident laser beams. These power losses increase with the concentration of plasmonic nanocapsules, shielding efficiencies close to 50%.

These results evidence the ability of these hollow structures to perform as light concentrators. A fair compromise between a high optical density and the preservation of visible light transmittance can be achieved.

Promover o desenvolvemento tecnolóxico, a innovación e unha investigación de calidade.
FEDER. Unha maneira de facer Europa. Centro Singular de investigación de Galicia 2016-2019

Oral Communication (ST10)

Role of TRPC and GLUD channels in MGLU1/5-GATED current in dopamine neurons

Rivas-Ramirez P, Hepp R, Hay A, Marti F, Faure P, Lambolez B, Tricoire L

Neuroscience Paris Seine, CNRS, INSERM, Sorbonne Université, Paris, France

Abstract:

In several brain structures, synaptic activation of group I glutamatergic receptors (mGlu1/5) causes a slow excitatory postsynaptic current (sEPSC). Canonical transient receptor channel (TRPC) and the delta family of ionotropic glutamate receptors (GluD) are involved in this excitatory effect. We previously have shown in mouse dopamine (DA) neurons that GluD1 is activated by metabotropic glutamate receptor (mGlu1/5), and that blocking GluD1 channel causes an inhibition of the sEPSC and impairs the burst firing of DA neurons. Thus, the relative contribution of TRPC and GluD channels has still to be determined. Using single cell RT-PCR on mouse DA neurons of the substantia nigra compacta (SNc), we observed that TRPC6 is the most highly expressed TRPC channel. We observed that mGlu1 co-precipitates with both GluD1 and TRPC6 but TRPC6 does not interact with GluD1. We then examined the contribution of TRPC in the sEPSC evoked by high frequency electrical stimulation of glutamatergic fibers using whole cell patch-clamp recording of SNc DA neurons in acute slices. The sEPSC amplitude was unaffected by the bath application of TRPC general blocker SKF96365, the TRPC3 preferring blocker Pyr3 and by the TRPC6 preferring blocker SAR7334. The application of GluD blocker NASPM reduced the sEPSC amplitude. When we included a train of depolarization before inducing the sEPSC in order to facilitate mGlu1/5 activation and to increase intracellular calcium release, we observed an increase of sEPSC amplitude by 38 % but sEPSC was still unaffected by SKF96365 and largely inhibited by NASPM. Nevertheless, we found that sEPSC was abolished in DA neurons of TRPC6-KO mice. In vivo recordings on anesthetized TRPC6-KO mice indicated that burst firing of DA neurons was moderately reduced but not abolished as observed in GluD1-KO. We are currently developing cell-specific molecular approaches to block specifically TRPC6 or GluD1 channels to clarify their contribution in the sESPC.

Promover o desenvolvemento tecnolóxico, a innovación e unha investigación de calidade.
FEDER. Unha maneira de facer Europa. Centro Singular de investigación de Galicia 2016-2019

Oral Communication (ST11)

HIF-1α deficiency dysregulates lipid metabolism and increases susceptibility to Leishmania Donovani infection

Mesquita I[1,2], Ferreira C[1,2], Moreira D[1,2], Kluck GEG[1,2,3], Barbosa AM[1,2], Torrado E[1,2], Dinis-Oliveira RJ[4,5,6], Gonçalves LG[7], Berod L[8], Sparwasser T[8], Bodhale N[9], Saha B[9,10,11], Rodrigues F[1,2], Cunha C[1,2], Carvalho A[1,2], Castro AG[1,2], Estaquier J[12,13], Silvestre[1,2]

1. Microbiology and Infection Research Domain (MIRD), Life and Health Sciences Research Institute (ICVS), School of Medicine, University of Minho, Braga, Portugal. 2. ICVS/3B's–PT Government Associate Laboratory, Braga/Guimarães, Portugal. 3. Medical Biochemistry Institute, Federal University of Rio de Janeiro, Rio de Janeiro, Brazil. 4. Department of Public Health and Forensic Sciences, and Medical Education, Faculty of Medicine, University of Porto, Porto, Portugal. 5. Department of Sciences, IINFACTS - Institute of Research and Advanced Training in Health Sciences and Technologies, University Institute of Health Sciences (IUCS), CESPU, CRL, Gandra, Portugal. 6. UCIBIO-REQUIMTE, Laboratory of Toxicology, Department of Biological Sciences, Faculty of Pharmacy, University of Porto, Porto, Portugal. 7. Instituto de Tecnologia Química e Biológica António Xavier, Universidade Nova de Lisboa, Oeiras, Portugal. 8. Institute of Infection Immunology, TWINCORE, Centre for Experimental and Clinical Infection Research, A Joint Venture between the Medical School Hannover (MHH) and the Helmholtz Centre for Infection Research (HZI), Hannover, Niedersachsen, Germany. 9. National Centre for Cell Science, Pune, India. 10. Case Western Reserve University, Cleveland, Ohio, USA. 11. Trident Academy of Creative Technology, Bhubaneswar, Odisha, India. 12. Centre de Recherche du CHU de Québec, G1V 0A6, Université Laval, Québec, QC. 13. CNRS FR3636, Université Paris Descartes, Paris, France.

Abstract:

Intracellular pathogens manipulate host cell metabolism by altering the expression of various intracellular mediators such as Hypoxia Inducible Factor-1 alpha (HIF-1α). We demonstrate that myeloid cell-specific deletion of mouse HIF-1α - a critical regulator of myeloid cell function - increases susceptibility to Leishmania donovani infection by increased lipogenesis. The enhanced susceptibility is associated with sterol regulatory element-binding protein-1c (SREBP-1c)-induced transcription of lipogenic genes such as fatty acid synthase (FASN), and acetate-driven lipid accumulation in infected mHIF-1α-/- mouse macrophages. L. donovani-infected myeloid-restricted HIF- 1α-deficient mice develop hypertriglyceridemia and lipid accumulation in splenic and hepatic myeloid cells. Pharmacological inhibition of FASN or SREBP-1c significantly reduced the susceptibility to L. donovani infection. Macrophages from individuals with loss-of-function HIF1A gene polymorphisms have increased Leishmania amastigotes and concurrent lipid accumulation. Our data thus indicate that genetic deficiency of HIF- 1α is associated with increased lipid accumulation and thereby results in impaired host- protective anti-leishmanial functions of myeloid cells.

Promover o desenvolvemento tecnolóxico, a innovación e unha investigación de calidade.
FEDER. Unha maneira de facer Europa. Centro Singular de investigación de Galicia 2016-2019

Oral Communication (ST12)

Human induced Pluripotent Stem cell-lines (iPSc) generation as an in vitro model of hand ostheoarthritis

Castro Viñuelas, R.[1,2], Sanjurjo Rodríguez C[1,2,3], Piñeiro Ramil M[1,2], Rodríguez Fernández S[1,2], Hermida Gómez T[2], De Toro-Santos FJ[1,2], Blanco García FJ[2], Fuentes Boquete I[1,2], Díaz-Prado S[1,2]

1. University of La Coruña, Spain. 2. Biomedical Research Centre of La Coruña (INIBIC), Spain.
3. University of Leeds, United Kingdom.

Abstract:

Research results in the field of hand osteoarthritis (hOA) are limited, mainly due to the unavailability of samples and lack of animal models replicating the features of the human disease. Induced pluripotent stem cells (iPSc) are ideal tools for modelling hOA since they grow unlimitedly in culture and present chondrogenic differentiation potential.
To generate iPSc-lines from patients with hOA and healthy donors and evaluate their chondrogenic differentiation potential in order to use them as cellular models of hOA.
Fibroblasts from 3mm skin biopsies of patients with hOA and a healthy control were harvested. The transcriptional factors Oct4, Sox2, Klf4 and c-Myc were used for the reprogramming, which was performed by using Sendai virus vectors. Cells obtained were morphologically, phenotypically and functionally characterized. To evaluate weather these iPSc lines could be used as cellular model of hOA, presence of 10 single nucleotide polymorphisms (SNPs) within genes associated with hOA were studied by Sanger sequencing, and chondrogenic differentiation capacity of the iPSc-lines was tested histologically.
Embryonic stem cell-like colonies emerged in culture three weeks after fibroblasts reprogramming, which fulfilled the morphologic and phenotypical criteria to be considered pluripotent cells. Different allelic variants among the lines were observed in 5 out of the 10 genes studied. Furthermore, the “ill” iPSc-line showed worse chondrogenesis than the “healthy” iPSc-line, as shown by the micromasses collagen and proteoglycan content.
The generation of two iPSc-lines form patients with hOA is reported for the first time. The obtained iPSC-lines carry different allelic variants in genes associated with hOA. Also, they show differences in their chondrogenic differentiation capacity, proving their usefulness to model hOA in vitro, and to deeper study the role of these variants in the pathogenesis of hOA.

Promover o desenvolvemento tecnolóxico, a innovación e unha investigación de calidade.
FEDER. Unha maneira de facer Europa. Centro Singular de investigación de Galicia 2016-2019

Oral Communication (ST13)

RADAR: R package for RNA-Seq experiment design and analysis

Mendoza M, Canchaya CA

Biomedical Research Centre (CINBIO), University of Vigo, Spain.

Abstract:

RNA-Seq technology arose a decade ago to improve the transcriptome analysis, allowing to study expression levels of transcripts and their isoforms as well as to identify possible coding-sequence mutations. The deluge of information generated by RNA-Seq implies computational challenges (e.g lots of memory and processing time) and many software approaches have been developed to analyse efficiently RNA-Seq data in different computational platforms and programming languages. R is a powerful working environment and programming language for data analysis; nowadays, more than 10,000 packages have been developed for many different purposes (e.g. statistical analysis, big data management). Here, we present the first draft of a new R package for RNA-Seq experiment design and analysis in R (RADAR), which allows us to accomplish the whole RNA-Seq pipeline in R: from experiment design and statistical power estimation to final differential gene expression analysis or gene ontologies enrichment. Using RADAR, we were able to analyse the impact of sample size (number of replicates per group and number of reads) in possible differentially expressed genes in simulated disease-control experiments.

Promover o desenvolvemento tecnolóxico, a innovación e unha investigación de calidade.
FEDER. Unha maneira de facer Europa. Centro Singular de investigación de Galicia 2016-2019

Oral Communication (ST14)

Validation of a microfluidic device for the isolation and characterization of circulating tumor cells towards cancer progression monitoring in metastatic breast cancer

Lopes C[1,2], Piairo P[1,3], Corredeira P[3], Costa L[3], Diéguez L[1]

1.Medical Devices research group, Department of Life Sciences, INL- International Iberian Nanotechnology Laboratory, Braga, Portugal. 2.Department of Physics and Department of Biology, University of Minho, Braga, Portugal. 3.Instituto de Medicina Molecular – João Lobo Antunes, Faculdade de Medicina, Universidade de Lisboa, Lisboa, Portugal

Abstract:

Breast cancer is the second most common cancer worldwide and a leading cause of cancer-related mortality in women. Metastasis is the underlying cause of cancer-related mortality. To improve clinical diagnostic and therapeutic decisions, it's necessary to develop new blood-based biomarker detection strategies. Circulating tumor cells (CTCs) that escape the primary tumor into the bloodstream, exhibit great metastatic potential. CTCs in body fluids represent a real-time snapshot of the tumor burden and offer unique opportunity for minimally invasive sampling in cancer patients. A head to head comparison of a microfluidic device (RUBYchip®, PCT/EP2016/078406) for CTC capture based on cell size and deformability, against the only FDA-approved technology, CellSearch®, was performed. Whole blood samples from metastatic breast cancer patients were collected at baseline (before treatment) and at follow-up (~12 weeks of treatment).
To optimize the performance of the RUBYchip®, breast cancer cell lines, MCF-7, MDA-MB 435 and SKBR3 were used to spike 7.5mL healthy whole blood samples. Different flow rates were tested to assess the isolation efficiency. Double amount of blood specimens were collected in order to test both technologies in parallel. CellSearch® analysis was performed in the Liquid biopsy analysis unit of Santiago (IDIS). Further identification and phenotypical analysis of CTCs was achieved by immunostaining with antibodies against cytokeratin, HER2 and CD45. Results can elucidate and identify CTC subpopulations which could be differentially associated with patients' clinical outcome. Comparing these technologies allows to understand the prognostic value of this sensitive and standardized approach for CTC isolation and phenotypic characterization in breast cancer patients.
Implementation of CTCs in the clinical management of metastatic breast cancer is expected to assist in the monitoring of disease progression, improve patient stratification and prognosis.

Promover o desenvolvemento tecnolóxico, a innovación e unha investigación de calidade.
FEDER. Unha maneira de facer Europa. Centro Singular de investigación de Galicia 2016-2019

Poster Communications

Promover o desenvolvemento tecnolóxico, a innovación e unha investigación de calidade.
FEDER. Unha maneira de facer Europa. Centro Singular de investigación de Galicia 2016-2019

Poster Communication (PC1)

Plasmonic Supercrystals for SERS detection

García-Lojo D, Gómez-Graña S, Pastoriza-Santos I, Pérez-Juste J

Dpto. de Química Física y Centro Singular de Investigaciones Biomédicas (CINBIO), Universidade de Vigo, Vigo, Spain.

Abstract:

Microfluidic platforms allows generating a highly-ordered assembly of uniform gold nanoparticles inside their microchannels through the pervaporation of the solvent. Furthermore, the microfluidic approach enables the fabrication of uniform assemblies of any dimension or morphology. The resulting plasmonic devices could be used for the detection of analytes, even without affinity for gold nanoparticles.

Surface-enhanced Raman spectroscopy, SERS, is an advanced analytical technique that can be used for the ultrasensible detection of analytes since it offers orders of magnitude increases in Raman signals. It occurs at the surface of a plasmon surface mainly due to the presence of strong electromagnetic fields generated after the plasmon excitation. Moreover, this effect could be more intense in the case of hierarchical nanoparticles assemblies due to an antenna effect as demonstrated by recent simulations.

While the plasmonic substrates made by drop-casting show poor uniformity that limits their potential plasmonic applications, the microfluidic approach gives rise to platforms with highly uniform and intense SERS activity (being both key parameters to achieve quantitative analysis and low detection limits (LOD). Herein, we will show the fabrication and characterization of plasmonic platforms fabricated using Au octahedra synthesized through a wet chemical method. Besides, the sensing capabilities of the platforms will be analysed by investigating the SERS efficiency using different Raman active analytes. For instance, experiment performed with Crystal Violet showed a great LOD, lower than 100zM, which is several orders of magnitude lower than those found in the literature.

Promover o desenvolvemento tecnolóxico, a innovación e unha investigación de calidade.
FEDER. Unha maneira de facer Europa. Centro Singular de investigación de Galicia 2016-2019

Poster Communication (PC2)

Plasmonic nanocapsules as SERS tags for multiplex detection

Rodal-Cedeira S[1], Vázquez-Arias A[1], Bodelón G[1], Laporta A[2], Nuñez-Sánchez S[1], Polavarapu L[3], Bals S[2], Liz-Marzán LM[4,5,6], Pérez-Juste J[1], Pastoriza-Santos I[1]

1. Departamento de Química Física and CINBIO, Universidade de Vigo, Vigo, Spain; 2. EMAT, University of Antwerp, Antwerp, Belgium; 3. Department of Physics, Ludwig-Maximilians-Universität München, Munich, Germany; 4. Bionanoplasmonics Laboratory, CIC biomaGUNE, Donostia-San Sebastian, Spain; 5. Ikerbasque, Basque Foundation for Science, Bilbao, Spain; 6. CIBER de Bioingeniería, Biomateriales y Nanomedicina, CIBER-BBN, Donostia-San Sebastián, Spain.

Abstract:

The unique optical properties of noble metal nanoparticles, NPs, arise from the so-called localized surface plasmon resonances (LSPRs). LSPRs are strongly dependent of NPs shape, size or composition. A wide range of methods based on wet chemistry have been developed to synthesize NPs with tailored optical properties. Recently our group has developed a strategy involving galvanic replacement reaction coupled seeded growth to fabricate Au/Ag nanorattles [1]. This work opens new venues towards the shape control of multimetallic hollowed nanostructures beyond the morphology of sacrificial templates.

Surface-enhanced Raman scattering (SERS) is an ultrasensitive technique which relies on the enhancement of the Raman scattering signals of a certain molecule when is close to a plasmonic nanostructure. This technique also allows the indirect identification of target molecules through the use of SERS tags, Raman encoded NPs, which comprises a specific Raman reporter attached to metallic NPs and often surrounded by a protected shell [2].

Herein we propose a new synthetic route to fabricate a SERS tag based on a room-temperature galvanic reaction coupled seeded growth method using Ag nanospheres (NSs) as sacrificial templates. During the process different Raman reporters could be trapped inside the resulting hollow Ag@Au NSs. The SERS tags NPs have been characterized by TEM revealing that the morphology of the sacrificial template remained after the reaction . Besides, we have analysed the SERS performance of the tags. This approach allowed us to codify the plasmonic particles with a library of Raman active molecules leading to the formation ultrasensitive SERS-encoded nanoparticles.

References:
[1]. L. Polavarapu et al., J. Am. Chem. Soc. 2016, 138, 11453.
[2]. Lingxin Chen et al., Chem. Rev. 2013,113, 1391.

Promover o desenvolvemento tecnolóxico, a innovación e unha investigación de calidade.
FEDER. Unha maneira de facer Europa. Centro Singular de investigación de Galicia 2016-2019

Poster Communication (PC3)

A new seeded growth methodology based on Iron (II) for the synthesis of noble metal nanoparticles

Fernández-Lodeiro C[1], Fernández Lodeiro J[2], Lodeiro C[2], Pérez-Juste J[1], Pastoriza-Santos I[1]

1. Departamento de Química Física y CINBIO, Universidade de Vigo, Vigo, Spain; 2. Bioscope Group. LAQV@REQUIMTE. Chemistry Department. Faculty of Science and Technology, Lisbon, Portugal.

Abstract:

Metallic nanoparticles (NPs) have nowadays many applications from sensing or catalysis to biomedical approaches. The huge versatility of these materials is mainly based on their optical properties which can be tuned controlling their size, shape and composition. In any case the synthesis of noble metal nanoparticles with narrow size distribution is still quite challenging. Spherical nanoparticles of different metals are an example of this. For instance, the synthetic procedure to obtain monodisperse gold nanoparticles needs tight temperature control, several synthetic steps and/or the use of different surfactants and polymers.

This work opens up a new synthetic route to obtain spherical nanoparticles with different composition (Au, Au@Ag and Au@Pd) with narrow size distributions based on a seeded growth and using Fe(II) as mild reducing agent.

Taking advantage of the well-known seeded growth methodology and the controlled addition of reagents through syringe pumps, different metal nanoparticles were synthesized. Small gold NPs were used as seeds and the overgrown with gold, silver or palladium has been done due to the ability of iron (II) to reduce their corresponding metal salts precursors. Through the careful control of the Au seed to metal salt concentrations ratio it is possible to nicely tune the final particle size.

Interestingly, the proposed methodology give rise to particles stabilized by citrate ions which can be easily exchanged with different molecules (such as proteins or thiolated molecules) expanding the potential applicability of the particles in a number of fields.

In summary, we propose a new methodology that enables fast synthesis of different metal nanospheres, at room temperature, with size control in aqueous media.

Promover o desenvolvemento tecnolóxico, a innovación e unha investigación de calidade.
FEDER. Unha maneira de facer Europa. Centro Singular de investigación de Galicia 2016-2019

Poster Communication (PC4)

New SERS-based method for multiplex detection of phenol derivatives

Carreira-Casais A, Montes-García V, Pastoriza-Santos I, Pérez-Juste J

Universidade de Vigo, Vigo, Spain

Abstract:

Phenol and its derivatives are common pollutants in food and fresh water. Their presence could cause acute effects on human health and also long-term effects.
Also they can indicate contamination by other pollutants because they are metabolites of some pesticides such as carbaryl and naphthalene.
Surface Enhanced Raman Spectroscopy (SERS) is a powerful technique that is gaining increase attention in last years in sensing field due to its very high sensitivity and selectivity with almost no need of sample preparation . In this context, we propose a SERS-based sensor for the multiplex detection of phenol and different phenol derivatives
The proposed method is based on the SERS-based detection of the product resulting from the chromogenic reaction between phenol or its derivatives with the Gibbs reagent, 2,6-dichloroquinone-4-chloroimide, in basic medium to form coloured indophenols or indophenolates employing phenol, o-cresol and 1-naphthol. It was employed a 633 nm laser lane and glass substrates covered layer by layer with 60 nm gold nanospheres.
This methodology allow us to differenciate between this three phenol derivatives and its mixtures in the µM regime employing principal components analysis (PCA). Also, this method allow us to quantify the concentration of each separate phenol derivative in a range between 0.25 µM and 10µM .
We can conclude that employing this technique could be really useful for the detection of phenol derivatives in fresh water and human samples. The multiplex detection of phenol derivatives in urine by SERS could be a first step to develop a new methodology to detect contamination by pesticides and other pollutants.

Promover o desenvolvemento tecnolóxico, a innovación e unha investigación de calidade.
FEDER. Unha maneira de facer Europa. Centro Singular de investigación de Galicia 2016-2019

Poster Communication (PC5)

SERS phenotyping of single cancer cells in microdroplets

Oliveira K[1,2], Teixeira A[1], Lopes C[1,3], Piairo P[1,4], Abalde-Cela S[1], Diéguez L[1]

1. International Iberian Nanotechnology Laboratory (INL), Braga, Portugal. 2. Universidade Nova de Lisboa, Campus da Caparica, Portugal. 3. Department of Physics and Department of Biology, University of Minho, Campus de Gualtar, Braga, Portugal. 4. Instituto de Medicina Molecular, Faculdade de Medicina, Universidade de Lisboa, Lisbon, Portugal

Abstract:

In the context of personalised medicine, the analysis of single-cells is key in order to understand the origin and evolution of cancer to provide an accurate prognosis. Microfluidics and microdroplets offer a perfect isolation environment for the study of single-cells. However, due to the small volumes handled in microfluidic devices, it is necessary to couple this technology to an ultrasensitive detection technique. The combination of surface-enhanced Raman scattering (SERS) spectroscopy with microfluidics offers a great potential for the development of automated and sensitive diagnostic platforms. Herein, SERS and droplet microfluidics were combined towards the analysis of single cancer cells. Initially, a set of microdroplet devices allowing droplet generation, single-cell encapsulation and storage were designed and optimised to achieve a generation of droplets with 80 µm of diameter. The capability of these devices as SERS sensing platforms was tested using gold nanostars, which were codified with Raman reporters and functionalised with antibodies for the proteins of a specific cell line. Following, single cancer cells were encapsulated together with the nanoparticles recognising specific membrane receptors expressed at cancer cells. More specifically, for the proof-of-concept of this technology, EpCAM was used for the targeting of MDA-MB-435 cancer cell line. Finally, those cells were characterised in the microfluidics platform using Raman spectroscopy. As a result, it was possible to identify the EpCAM expression on the MDA-MB-435 down to the single cell level. In this way, a phenotypic study at the single cell level was made for a breast cancer model line. The development of these platforms intends to pave the way towards a more personalised handling of cancer diagnosis and monitoring, in a reproducible, automated and fast way.

Promover o desenvolvemento tecnolóxico, a innovación e unha investigación de calidade.
FEDER. Unha maneira de facer Europa. Centro Singular de investigación de Galicia 2016-2019

Poster Communication (PC6)

Changes in lung transcriptomic profile driven by a novel tuberculosis nanovaccine

Martínez-Pérez A[1], Ana Igea A[1], Estévez-Martínez O[1], M Ferreira C[2], Reljic R[3], Singh M[4], G Castro A[2], Torrado E[2], González-Fernández A[1]

1. Immunology. Biomedical Research Centre (CINBIO) (Singular Centre of Research), Galicia Sur Health Research Institute (IISGS), University of Vigo. Vigo, Spain. 2. Life and Health Sciences Research Institute (ICVS), School of Medicine, University of Minho, Braga, Portugal, and PT Government Associate Laboratory ICVS/3B's. 3. St George's Medical School, University of London, London SW17 0RE, UK. 4. Lionex GmbH, Braunschweig, Germany.

Abstract:

The mechanisms underlying protection against Mycobacterium tuberculosis (Mtb) infection remain unclear. Prime vaccination with Bacille Calmette Guerin (BCG) vaccine followed by boosting with novel vaccines has emerged as promising strategy. In this regard, the intranasal administration of a new vaccine composed by nanoparticles and fusion protein containing three Mtb antigens, has been shown to enhance protection against Mtb, reducing lung colony formation units (CFUs) by a further 0.6 log on average compared with BCG alone (Hart et al, Mol Ther, 26(3):822-833, 2018).
In this study, we aimed to define the transcriptomic profile generated by this vaccine with the ultimate goal of identifying correlates of protection against Mtb infection.
Materials and methods: Mice were vaccinated with BCG following by two boosts two weeks apart with the nanovaccine termed Nano-1. Mice were sacrificed at different time-points after the last boost, and lung parenchyma and bronchoalveolar lavage cells were analyzed by RNA-Sequencing.
Gene expression analysis revealed a unique profile of differentially expressed genes in protected mice at short-term, involving several immune-related pathways.
The results obtained in this study offer new insights of the cell transcriptional profile related with TB protection, which may be useful for the design of novel tuberculosis vaccines and a deeper characterization of the immune response to Mtb.

Promover o desenvolvemento tecnolóxico, a innovación e unha investigación de calidade.
FEDER. Unha maneira de facer Europa. Centro Singular de investigación de Galicia 2016-2019

Poster Communication (PC7)

Shaping multicomponent iron oxide nanocrystals for magnetic separation applications

Testa-Anta M[1], Rivas-Murias B[1], Rodríguez-González B[2], Salgueiriño V[1]

1. Departamento de Física Aplicada, Universidade de Vigo, Spain. 2. CACTI, Universidade de Vigo, Spain

Abstract:

Owing to their low cytotoxicity and huge platform of magnetic properties displayed, iron oxide nanoparticles have drawn a lot of attention in the field of biomedicine, where they are ubiquitously used for magnetic guidance, drug delivery and as contrast agents for imaging.1 Their suitability for each of these bio-related applications relies on different parameters such as the coercive field, saturation magnetization and magnetic susceptibility, all of them related to the effective anisotropy ruling the nanocrystals magnetic behavior. Hence, a rational design of iron oxide nanocrystals for a particular bio-application necessarily involves exerting control on the magnetic anisotropy, fact that can be assessed in terms of their size, morphology, surface functionalization or, more interestingly, by considering multicomponent systems.2 Along these lines, the relative distribution and physical interactions between the different phases can provide a unique scenario that may open the door to an improved performance.3

Herein, we report a one-pot synthesis of core-shell FeO@Fe3O4 nanoparticles by thermal decomposition in oleic acid. Given the high temperature of the reaction and the particular synthetic conditions, the attained nanostructures display an octopod-like geometry and are endorsed with (i) a high saturation magnetization, and (ii) a negligible coercivity. These two factors, jointly with their intermedium size, prevent magnetic aggregation and lead to an enhanced magnetophoretic mobility, rendering them optimal for magnetic separation and drug delivery applications.

References:

1. Q. A. Pankhurst, J. Connolly, S. K. Jones, J. Dobson, J. Phys. D: Appl. Phys., 2003, 36, 167-181
2. M. Testa-Anta, S. Liébana-Viñas, B. Rivas-Murias, B. Rodríguez-González, M. Farle, V. Salgueiriño, Nanoscale, 2018, 10, 20462-20467
3. M. Testa-Anta, M. A. Ramos-Docampo, M. Comesaña-Hermo, B. Rivas-Murias, V. Salgueiriño, Nanoscale Adv. (in press, 2019)

Promover o desenvolvemento tecnolóxico, a innovación e unha investigación de calidade.
FEDER. Unha maneira de facer Europa. Centro Singular de investigación de Galicia 2016-2019

Poster Communication (PC8)

Magnetic manipulation of artificial swimmers for potential intracellular applications

Ramos-Docampo MA[1], López-Fanarraga M[2], Salgueiriño V[1]

1. Departamento de Física Aplicada, Universidade de Vigo, Vigo, Spain. 2. Departamento de Biología Molecular, Universidad de Cantabria-IDIVAL, Santander, Spain.

Abstract:

Nano- and microswimmers are becoming a powerful and versatile option towards their use in biomedical applications. Among the mechanisms that swimmers may use to gain motion, one can distinguish between self-propulsion and external stimuli. Magnetic, electric, and thermal fields are indeed examples of the use of physical stimuli to direct locomotion. Specifically, many magnetic nanomaterials, such as transition metal ferrites, have been demonstrated to be not harmful when interacting with cells or tissues, and they are finally eliminated from the biological systems. Therefore, decorating swimmers with magnetic nanoparticles may result in a clever strategy to externally guide to or retain the swimmers in specific locations, without damaging the surrounding tissue.[1]

Hence, we herein present i) the assembly of a magnetic swimmer decorated with magnetite nanoparticles; ii) the mobility analysis of the swimmers in three different media; iii) the swimmer-cell internalization mechanisms; and iv) the magnetic manipulation of the swimmers inside living cells.

The results indicated that these swimmers have exhibited a good response to the externally-applied magnetic field, and eventually self-assembled into chain-like structures. The length of the chains may be tuned by varying the viscosity of the media. Additionally, the mobility of these assemblies was evaluated in three bio-relevant media, as a proof-of-concept for guaranteeing their motion in complex environments. With the aim of studying the magnetic manipulation in living organisms, the swimmers were tested in cells. Owing to the different internalization mechanisms, the swimmers allocated either in the cytoplasm or inside endo-lysosomes. This fact of swimmers interacting with various architectures inside cells in a controlled manner might render potential advantages in bio-related applications, such as magnetic cell sorting or intracellular manipulation.

Reference:
1. Schattling et al. ACS Nano 2017, 11, 3973.

Promover o desenvolvemento tecnolóxico, a innovación e unha investigación de calidade.
FEDER. Unha maneira de facer Europa. Centro Singular de investigación de Galicia 2016-2019

Poster Communication (PC9)

Multiple testing procedures for discrete uniform and homogeneous tests with application to genomics

Soage JC[1,2], Cousido-Rocha M[1,2], de Uña-Álvarez J[1,2], Döhler S[3]

1. SiDOR Research Group, University of Vigo, Spain 2. University of Vigo, Spain 3. University of Applied Sciences Darmstadt, Germany

Abstract:

The statistical analysis of data from genome-wide studies is non-trivial due to their complexity. This analysis often involves data on several small groups of individuals (e.g. two tumor groups) for which a large number of variables (e.g. gene expression levels) are measured.
In this context, the researcher usually aims to compare the distribution of each variable in the different groups. For this goal a large number of hypotheses need to be tested simultaneously leading to large scale P-values, which can be discrete distributed. We focus on the homogeneous discrete uniform P-values which arise in many application where, for example, permutation tests are used.
The multiple comparison procedures availables in the literature are not suitable for homogeneous discrete uniform P-values. Hence, we adapt the q-value method of Storey and Tibshirani (2003), originally proposed for continuous P-values, to take this type of discreteness into account.
The conclusions derived from simulations are that the adaptations of the q-value approach for discrete P-values improve the original q-value procedure.
The real data illustration is based on a microarray of hereditary breast cancer, which consists of 3170 logged gene expression levels measured on 7 and 8 patients with breast tumors having BRCA1 and BRCA2 mutations, respectively. We are interested in determining which genes are not equally distributed in both types of tumors. Hence, we test the 3170 hypotheses using different two-sample tests. Some of the tests lead to homogeneous discrete uniform P-values. In such case the correct performance of the q-value methods adapted to the discreteness is shown. All the q-value methods are implemented in the user-friendly DiscreteQvalue package of the free software R which makes it easy to apply in practice.

Reference:
Storey, J and R Tibshirani (2003). Statistical significance for genomewide studies. Proc. Natl. Acad. Sci. 100, 9440-9445.

Promover o desenvolvemento tecnolóxico, a innovación e unha investigación de calidade.
FEDER. Unha maneira de facer Europa. Centro Singular de investigación de Galicia 2016-2019

Poster Communication (PC10)

New quantitative method for image analysis of haematoxylin and eosin (HE) gonads histological slides

Campoy-López P, de la Torre E, Mantilla-Aldana L, Beiras R

University of Vigo, Vigo, Spain

Abstract:

Image analysis is a powerful tool used in different science fields such as medicine, computer, engineer, life and nature. This instrument gives information present in pictures using mathematical algorithms corroborate scientifically. In natural sciences is common using the image analysis in histological samples, but it is less used as tool to obtain gonad information. The typical gonad analysis in marine animal are: gonadosomatic index using weights and maturation or condition stage by visual analysis. The aim was implementing a new quantitative method for image analysis of haematoxylin and eosin (HE) gonads histological slides using the pixel colour variations in different maturation stages by software tools such as Color Deconvolution and CellProfiler. The structure of the echinoderms gonads, in terms of cellular types, makes application of pixel analysis feasible. The HE stains shows two cell types with a different pattern of colour, the germinal cells are stained in violet given their basophilic nature, whereas the somatic cells are shown in pink by eosinophilic nature. The information of colour were transformed in a binary format and binary images obtained were quantified by the Cell Profiler software creating a pipeline effect and using the Measure Image Area Occupied tool. This pixel's information was used to calculate two indices. In the first (PI1) only the ratio between the violet pixels in relation to violet plus pink was computed, while in the second (PI2) white pixels (empty space or lumen) were also taken into account. A total of 167 gonad samples were analysed: 102 females and 65 males. In both cases the evolution of the PI fit well to a bell-shaped Gaussian function with r2 ranging 0.937-0.999. However, the shape of the curve is different between sexes. In females the PI values markedly increase until stage IV, whereas in males the shape of the curve is flatter and more symmetrical, and similar PI values are recorded for stages II, III and IV.

Promover o desenvolvemento tecnolóxico, a innovación e unha investigación de calidade.
FEDER. Unha maneira de facer Europa. Centro Singular de investigación de Galicia 2016-2019

Poster Communication (PC11)

Classification of cardiac abnormalities in ECGs: wave delineation and generalized additive models

Mondelo V[1], Roca-Pardiñas J[2], Lado MJ[1], Méndez AJ[1]

1.Department of Computer Science, ESEI, University of Vigo, Spain. 2. Department of Statistics and Operational Research, University of Vigo, Spain

Abstract:

Cardiovascular diseases are important causes of death [1]. Computer systems working over the ECG (recording of the electrical activity of the heart) are devoted to help clinicians in early diagnosis of these diseases [2].
To develop an algorithm to classify cardiac abnormalities.
Forty-three ECGs from patients suffering from bradycardia (9), tachycardia (13), myocardial infarction (11), ischemia (7), ventricular hypertrophy (8), and Wolff-Parkinson-White Syndrome (3) were considered; the method was applied in a 3-step process:
1) Heartbeat detection: a modified version of the Pan-Tompkins algorithm was used [3].
2) Waves delineation: a basal beat (optimized beat) was generated for the detected beats in several portions of the original signal. Artefacts removal, wavelet transform application, and delineation of the beat waves (QRS complex, P and T waves) was performed for each ECG.
3) Diagnosis: generalized additive models (GAM) were used to classify abnormalities. Values such as RR distance (distance between consecutive beats), and wave duration and amplitude were computed, and considered as input variables of the model. The final model was constructed by selecting the GAM which yielded the greatest probability function, maximizing the AUC (area under the ROC curve) values.
The following AUC values were obtained: bradycardia: 0.93; tachycardia: 0.95; myocardial infarction: 0.72; ischemia: 0.74; ventricular hypertrophy; 0.69; and Wolff-Parkinson-White Syndrome: 0.78.
Results obtained show promising disease detection capabilities, making it a good candidate to assist clinicians in diagnosis tasks.

References:
[1] World Health Organization: http://www.who.int/
[2] Martis RJ et al. (2014) Current methods in electrocardiogram characterization. Comput. Biol. Med. 48:133-149.
[3] Mondelo V et al. (2017) Detection of heart beat positions in ECG recordings: A lead-dependent algorithm. JISEM 2:13.

Poster Communication (PC12)

Construction of percentile curves of the Galician child population

Arias Vázquez M[1], Santiago Pérez MI[2], Iglesias Pérez MC[3]

1. Master técnicas estadísticas e investigación operativa (USC), Santiago de Compostela, Spain. 2. Servicio de epidemiología de la consellería de sanidad. 3. Universidade de Vigo, Vigo, Spain.

Abstract:

The objective of this work was the construction of growth percentiles of the Galician child population. These curves are useful both in the clinical setting and in the epidemiological field, where percentiles are used as reference values for the classification of individuals.

There are several reference percentile curves, both internationally and nationally. Among the first were published by the WHO in 2007, and the International Working Group on Obesity, developed by Cole and Green for the classification of overweight, obesity (2000) and low weight (2006). In Spain, the study by the Orbegozo Foundation stands out, the percentile curves are used in pediatric health controls.

In 2013, the General Directorate of Public Education carried out an anthropometric study of the Galician school population from 6 to 15 years old, with measurements of 7,438 representative students of that population. These data are used in this work to estimate the percentile curves of weight, height, body mass index (BMI), waist circumference and hip perimeter, based on age, and boys and girls separately.

This work focuses on the generalized additive models of location, scaling and shape (GAMLSS), which generalize the estimation methods of percentiles: the LMS method of Cole and Green (1982), which assumes a normal Box-Cox distribution with 3 parameters, and the BCPE or LMSP method of Rigby and Stasinopoulos (2004), which assumes a Box-Cox Power Exponential distribution with 4 parameters. Cubic and penalized splines were used to model the dependence of each of them. The estimation and diagnosis of the models were carried out with the gamlss library of R.

Promover o desenvolvemento tecnolóxico, a innovación e unha investigación de calidade.
FEDER. Unha maneira de facer Europa. Centro Singular de investigación de Galicia 2016-2019

Poster Communication (PC13)

Rare ABCC8 variants identified in Spanish pulmonary arterial hypertension patients

Lago-Docampo M[1], Tenorio J[2], Pérez-Olivares C[3], González-Hernández I[4], Escribano-Subías P[5], Pousada G[6], Baloira A[7], Arenas M[8], Lapunzina P[9], Valverde D[1]

1. Departamento de Bioquímica, Genética e Inmunología, Facultad de Biología, Universidad de Vigo, Vigo, Spain. 2. Instituto de Investigación Sanitaria Galicia Sur (IIS Galicia Sur), SERGAS-UVIGO, Vigo, Spain. 3. Centro de Investigaciones Biomédicas (CINBIO), Vigo, Spain. 4. Instituto de Genética Médica y Molecular (INGEMM), Hospital Universitario La Paz-IdiPaz, Universidad Autónoma de Madrid, Madrid, Spain. 5. Centro de Investigación Biomédica en Red de enfermedades Raras (CIBERER), Instituto de Salud Carlos III, Madrid, Spain. 6. Servicio de Cardiología, Hospital 12 de Octubre, Madrid, Spain. 7. Unidad Multidisciplinar de Hipertensión Pulmonar, Servicio de Cardiología, Hospital Universitario 12 de Octubre, Madrid, Spain. 8. Centro de Investigación Biomédica en Red de Enfermedades Cardiovasculares (CIBERCV), Instituto de Salud Carlos III, Madrid, Spain. 9. Servicio de Neumología, Complejo Hospitalario de Pontevedra, Pontevedra, Spain.

Abstract:

Pulmonary Arterial Hypertension (PAH) is a rare and fatal disease consisting in the obliteration of the pulmonary precapillary arteries, leading to right heart failure and death. Next Generation Sequencing has allowed us to detect hundreds of variants related to PAH, but it has also increased the number of Variants of Unknown Significance (VUS) that we find, in order to give a correct diagnosis to patients, VUS functional effect must be analyzed.

We used targeted panel sequencing with a 21 genes custom panel (HAP v1.2) in 318 PAH patients from the Spanish registry (REHAP) and detected ten changes in the novel candidate gene ABCC8. We carried out a functional analysis by minigene assay to evaluate splicing variants (8/10). We then used UCSC genome browser to look for regulatory motifs. Lastly, we used protein modeling by homology (Phyre2) to evaluate the pathogenicity of the changes at protein level assessing its stability with MODELLER.

After validation, we identified ten variants in the ABCC8 gene that had never been related to PAH: p.(Glu100Lys), p.(Ala726Thr) and p.(Val1080Ile) were classified as VUS. p.(Val477Met), p.(Thr548Met), p.(Gln808Lys), c.2694+1G>A and p.(Glu1326Lys) were classified as likely pathogenic. Lastly, p.(His1097ProfsTer16) and p.(Asp1132Asn) were classified as pathogenic. Minigenes confirmed the pathogenicity of p.(Asp1132Asn) inducing an exon skipping, and the correct processing of p.(Glu100Lys) and p.(Thr548Met). However, they were inconclusive for five variants as none of the encoded exons were transcribed correctly. Protein modeling revealed that amino acid changes would not alter protein stability while the skipping of exons 20, 27 and 32 would yield unstable proteins.

We identified ten variants in ABCC8, confirmed experimentally the pathogenicity of p.(Asp1132Asn). Protein stability analysis allowed us to predict the possible outcomes of the unconfirmed splicing variants.

Promover o desenvolvemento tecnolóxico, a innovación e unha investigación de calidade.
FEDER. Unha maneira de facer Europa. Centro Singular de investigación de Galicia 2016-2019

Poster communication (PC14)

Role of subchondral Mesenchymal Stromal Cells in the osteoarthritis pathogenesis

Sanjurjo-Rodriguez C[1,2,3], Crossland R[2], Baboolal T[3,4], Reis M[2,5], Burska A[3,] Ponchel F[3], El-Jawhari J[3], Pandit H[3,4], McGonagle D[3,4], Wang X[2] , Jones E[3]

1. Cell Therapy and Regenerative Medicine group, University of A Coruña, Biomedical Sciences, Medicine and Physiotherapy department, CIBER-BBN, Institute of Biomedical Research of A Coruña (INIBIC)-Centre of Advanced Scientific Researches (CICA), A Coruña, Spain. 2. Institute of Cellular Medicine, Newcastle University, UK. 3. Leeds Institute of Rheumatic and Musculoskeletal Medicine, University of Leeds, Leeds, UK. 4. NIHR Leeds Musculoskeletal Biomedical Research Unit, Leeds Teaching Hospitals NHS Trust, Leeds, UK 5. Department of Pediatrics, Harvard Medical School, Boston, MA, USA

Abstract:

Recent studies described that in osteoarthritis (OA) there is an abnormal recruitment and osteogenic commitment of MSCs. Thus, subchondral bone MSCs could be implicated in the failure of cell therapy in OA patients. As OA severity is often associated with an abnormal movement and load distribution on the medial (Med) part of the knee, the aim was to determine differences in subchondral bone MSC topography and gene expression (GE) between Med and lateral (Lat) femoral condyles (FC). Also, we investigated the miRNA profiling of Med OA MSCs compared with healthy MSCs.

OA MSCs were obtained from FC of total knee replacement patients and were extracted from subchondral bone after digestion and sorted using the CD271+CD45- phenotype for GE analysis. FC were decalcified for histological evaluation. MSCs from healthy donors were isolated from surplus to hematopoietic stem cell transplantation by density gradient centrifugation using Lymphoprep and culture expanded for miRNA profiling in comparison with culture expanded medial MSCs.

Med FC presented higher OARSI score for degree of cartilage damage compared to Lat FC, and sclerotic subchondral bone area was higher in the Med FC (64.3±7.0% vs 27.6±7.4% area in Lat FC). CD271+ cells in Med and Lat FC did not show differences in topography or numbers. MSCs presented similar growth rates and trilineage capacities. Three genes were upregulated in Med FC CD271+ MSCs: GREM1 (lateral MSCs below detection), PTHLH (2.4-fold, p=0.02) and STMN2 (10.5-fold, p=0.02), all implicated in osteogenic differentiation and mineralisation. miRNA profiling showed differentially expressed miRNAs in OA-MSCs compared with healthy MSCs.

No major differences were found between Med and Lat FC in terms of MSC number and topography. There was difference in miRNA expression, and upregulation of genes implicated in osteogenesis and mineralisation in Med FC MSCs. These results can implicate local subchondral bone MSCs in knee OA pathogenesis.

Promover o desenvolvemento tecnolóxico, a innovación e unha investigación de calidade.
FEDER. Unha maneira de facer Europa. Centro Singular de investigación de Galicia 2016-2019

Poster Communication (PC15)

Corneal cryopreservation: and endothelial cell viability and histomorphological study of cornea

Rodríguez-Fernández S[1,5], Álvarez-Portela M[2], Rendal-Vázquez E[3], Montero-Salinas A[4], Piñeiro-Ramil M[1,5], Castro-Viñuelas R[1,5], de Rojas MV[2], Sánchez-Ibáñez J[3], Fuentes-Boquete IM[1,5], Díaz-Prado S[1,5]

1.Instituto de Investigación Biomédica de A Coruña (INIBIC), A Coruña, Spain. 2. Servicio de Oftalmología, Complejo Hospitalario Universitario de A Coruña (CHUAC), SERGAS, A Coruña, Spain. 3. Unidad de Criobiología-Banco de Tejidos, CHUAC, SERGAS, A Coruña, Spain. 4. Oficina de Coordinación de Trasplantes, CHUAC, SERGAS, A Coruña, Spain. 5. Grupo de Terapia Celular y Medicina Regenerativa, Departamento de Fisioterapia, Medicina y Ciencias Biomédicas, Facultad de Ciencias de la Salud, Universidade da Coruña (UDC), A Coruña, Spain.

Abstract:

Preliminary evaluation of four cryopreservation protocols, attending to endothelial cellularity, endothelial cell viability, and to the histology of whole corneas.
12 corneas were cryopreserved using the protocols below: Protocol 1: slow cryopreservation (SC) with albumin and DMSO in 199 media; Protocol 2: SC with DMSO in 199 media; Protocol 3: fast cryopreservation (FC) with VS55 media, and Protocol 4: FC with DP6 media. An assay with LIVE/DEAD imaging kit and Hoechst was realised to evaluate cellularity and endothelial cell viability. A Masson trichrome staining was preformed to the histological evaluation.
Endothelial cellularity was huge in all corneas (in descending order, Protocols 4, 3, 1 and 2). Regarding cell viability, corneas which were cryopreserved with Protocol 1 showed the high viable cells rate (66,79%), while, with the other three protocols there were non-viable cells (Protocols 2, 3 and 4). Cryoinjuries were observed in endothelium detachment in corneas cryopreserved with Protocols 2, 3 and 4. Moreover, corneas cryopreserved with Protocols 1, 3 and 4 showed holes among collagen fibres of stroma. In all cryopreservation groups, epithelium was thinner, and some cells were lost.
Cryopreservation with any of the four protocols allow a high endothelial cellularity after thawing. Fast cryopreservation offers the best result in cellularity. Despite of all, endothelial cell viability is affected by fast cryopreservation while slow cryopreservation retains some cell viability. Endothelium, epithelium and stroma are three sensitive corneal layers that suffer cryoinjuries, in special, when the fast cryopreservation is carried out.

Promover o desenvolvemento tecnolóxico, a innovación e unha investigación de calidade.
FEDER. Unha maneira de facer Europa. Centro Singular de investigación de Galicia 2016-2019

Poster Communication (PC16)

A new model to estimate covariate dependent bivariate reference regions for two laboratory tests. The case of fasting plasma glucose and glycated haemoglobin

Lado-Baleato O[1], Roca-Pardiñas J[2], Cadarso-Suárez C[1], Gude F[3]

1. Unit of Biostatistics, Department of Statistics, Mathematical Analysis, and Optimization, Universidade de Santiago de Compostela, Spain. 2. Department of Statistics and Operations Research, SiDOR Research Group, University of Vigo, Spain. 3. Clinical Epidemiology Unit, Complejo Hospitalario Universitario de Santiago de Compostela, Spain.

Abstract:

Clinical laboratory tests are an everyday procedure in disease prevention, diagnose and control. With every test result, clinical laboratories provide a comparator value to help the clinician to contextualize results. The comparator values are often referred to as the reference interval, usually defined as the set of values in which 95% of the normal healthy population falls. In case of more than one test for a disease, each test result is usually compared with its respective reference interval separately. This procedure considers the test results as independent, ignoring that usually are highly correlated. This is the Diabetes mellitus diagnosis problem, which is based on two tests, fasting plasma glucose (FPG) and glycated hemoglobin (HbA1c). Currently, a patient is diagnosed with diabetes if FPG>125 mg/dL or HbA1c>6.50%, even though both test results are correlated in the normal healthy population and show a great variation between age groups. Thus, we propose that an agespecific bivariate reference region should be more appropriate for diabetes diagnose.

In this comunication, we present a model to obtain a bivariate reference region conditioned on covariates. The proposed model has no parametric restrictions except it considers the relationship between both responses in terms of linear correlation. However, the model offers good coverages of the bivariate data points even in datasets considering non-gaussian responses and asymmetric dependence. Using a sample of the general healthy population taken in A Estrada municipality (n=1516) we estimated an age-dependent bivariate reference region for FPG and HbA1c. The estimated region showed a coverage of the joint distribution close to the nominal level, and when applied to patients already diagnosed from diabetes, it identified them as “abnormal”. The obtained bivariate reference region can be easily displayed using an R based application, being a new tool for understanding glycemic tests from a multivariate perspective.

Promover o desenvolvemento tecnolóxico, a innovación e unha investigación de calidade.
FEDER. Unha maneira de facer Europa. Centro Singular de investigación de Galicia 2016-2019

Poster Communication (PC17)

Proteomics in Schizophrenia: a Gateway to Discover Potential Biomarkers of Psychoneuroimmune Pathways

Rodrigues-Amorim D[1], Rivera-Baltanás T[1], Rodriguez-Jamardo C[1], Vallejo-Curto MC[1], de las Heras E[1], Caballero A[1,2], Olivares JM[1], Spuch C[1]

1. Translational Neuroscience Research Group, Galicia Sur Health Research Institute. SERGAS-UVIGO, CIBERSAM, Spain.
2. Departement of Psychiatry, University of Santiago de Compostela, Spain.

Abstract:

Schizophrenia is a chronic, severe and disabling psychiatric disorder with a complex neurobiological pathophysiology that translates into an ambiguous aetiology. The lack of consensus regarding the pathogenesis of this ailment has increased the need to explore new research lines in this regard. Advances in "omics" sciences, such as proteomics, promotes new approaches to better understand the molecular basis of schizophrenia and identify potential biomarkers. This research makes use of proteomics data to discover possible analytes associated with psychoneuroimmune signalling pathways in schizophrenia.

A comparative proteomic analysis was performed on the plasma of 45 patients with schizophrenia and 43 healthy subjects, and label-free liquid chromatography-tandem mass spectrometry (LC-MS/MS) was used to identify and quantify proteomes. Protein immunoblot analyses were carried out to evidence the proteomics results.

Upon quantification, the analysis revealed a significant reduction in the levels of glia maturation factor beta (GMF-β) (P=0.0256), the brain-derived neurotrophic factor (BDNF) (P=0.0008), and the 115-kDa isoform of the Rab3 GTPase-activating protein catalytic subunit (RAB3GAP1) (P=0.0002) in patients with schizophrenia as compared to healthy volunteers. Statistical significance was also founded between the groups of first-episode schizophrenia (FES) and chronic schizophrenia compared to controls concerning the levels of BDNF (P=0.0031; P=0.0131), 115-kDa isoform of RAB3GAP1 (P=0.0080; P=0.0020), respectively, and GMF-β levels in the chronic schizophrenia group (P=0.0453). Attractin, drebrin and RAB3GAP1 did not yield significant results, as well as, correlations of proteins levels with PANSS scores (P >0.05).

The GMF-β, BDNF, and 115-kDa isoform of RAB3GAP1 are associated with different biological mechanisms taking place in the central nervous system, thus making them potential biomarkers in schizophrenia.

Promover o desenvolvemento tecnolóxico, a innovación e unha investigación de calidade.
FEDER. Unha maneira de facer Europa. Centro Singular de investigación de Galicia 2016-2019

Poster Communication (PC18)

Bioinformatics Docker Images

Ferreira P[1,2], López-Fernández H[3,4,5], Duque P[1,2], Vieira CP[1,2], Fdez-Riverola F[3,4,5], Reboiro-Jato M[3,4,5], Vieira J.[1,2]

1. Instituto de Biologia Molecular e Celular (IBMC), Porto, Portugal. 2. Instituto de Investigação e Inovação em Saúde (I3S), Porto, Portugal. 3. ESEI - Escuela Superior de Ingeniería Informática, Universidad de Vigo, Ourense, Spain. 4. Centro de Investigaciones Biomédicas, Vigo, Spain. 5. SING Research Group, Universidad de Vigo, Spain.

Abstract:

Nowadays bioinformatics is one of the most important areas in modern biology.
Nevertheless, the use of scientific software is not always easy for researchers without an informatics background. This is mainly due to i) availability of the software for Linux operating systems only, since most researchers working on the life sciences use Windows or Macintosh operating systems; ii) the complexity of the installation, since scientific software often have many dependencies and some may be non-trivial to install. Docker images have many advantages such as i) easy to use since it only requires the installation of Docker; ii) can be deposited in public databases such as Docker Hub; iii) can be downloaded when needed and erased when no longer needed; iv) presents a user friendly management interface – Portainer.io; v) software invoked through the command line can be directly run in Linux and Windows platforms; vi) Docker images can be used in pipelines; and vii) all Docker images can be run using a VirtualBox Ubuntu image previously installed with Docker.
The Bioinformatics Docker Images Project (https://pegi3s.github.io/dockerfiles/) aims at providing Docker images for commonly used scientific software providing clear instructions on how to use such images and when appropriate providing test cases. For users wishing to use software with a graphical interface in Windows hosts, we provide a VirtualBox image with Docker installed. The main difference between this project and much larger projects such as Biocontainers is the detailed information that is given allowing researchers without a background in informatics to easily use these Docker images. At present, we provide images for about 30 scientific softwares, commonly used in genomics, transcriptomics, sequence analyses, and phylogenetics, and new images are constantly being added. This work is part of the BioData.pt project, the Portuguese distributed e-infrastructure for biological data and the Portuguese node of ELIXIR.

Promover o desenvolvemento tecnolóxico, a innovación e unha investigación de calidade.
FEDER. Unha maneira de facer Europa. Centro Singular de investigación de Galicia 2016-2019

Poster Communication (PC19)

Analysis of endothelin-1 (EDN-1) UTR regions

Solarat C[1], Lago-Docampo M[1,2,3], Baloira A[4], Valverde D[1,2,3]

1. Departamento de Bioquímica, Genética e Inmunología, Facultad de Biología, Universidad de Vigo, Vigo, Pontevedra, España. 2. Instituto de Investigación Sanitaria Galicia Sur (IIS-Galicia Sur), Pontevedra, España . 3. Centro de Investigaciones Biomédicas (CINBIO), Pontevedra, España . 4. Servicio de Neumología, Complexo Hospitalario Universitario de Pontevedra, Pontevedra, España

Abstract:

Pulmonary Arterial Hypertension (PAH) is a disease characterized by an increase of secretion and deregulation of Endothelin-1 (ET-1). This peptide is secreted by the endothelium of blood vessels and promotes vasoconstriction. We carried out the characterization of the UTR regions of endothelin-1gene (EDN-1), in order to determine common variations that may modulate disease outcome.
The analysis was carried out in 60 patients with different classes of PAH, testing a fragment of 2 kb for both UTR region. An in silico analysis was performed to evaluate binding transcription factors. Luciferase assay was done to evaluate in vitro the SNP influence in gene expression. Data revealed the presence of a deletion in the promoter region (rs397751713), while a transversion in the 3' UTR region was found (rs2859338). The distribution of the genotype frequencies in our PAH patients were: for rs397751713: A/A: 0.08; A/-: 0.27; -/-: 0.66; for rs2859338: A/A: 0.15; A/G: 0.60; G/G: 0.25. Variations are located in a KLF4 binding sequence and a vitamin D receptor binding sequence respectively. Both transcription factors are related to PAH development.
In conclusion, these SNPs in the UTR regions of EDN1 are related with gene expression levels, as we measured higher expression rates for patients with A/A and G/G genotype. Moreover, we hypothesized that this overexpression is due to the inability of KLF4 and vitamin D receptor to attach the target sequence and to regulate the expression of EDN1, as KLF4 is probe to avoid PAH when present and vitamin D is an anti-hypertrophic factor.

Promover o desenvolvemento tecnolóxico, a innovación e unha investigación de calidade.
FEDER. Unha maneira de facer Europa. Centro Singular de investigación de Galicia 2016-2019

Poster Communication (PC20)

Characterization of a CRISPR-Cas9 cellular model to Alström Syndrome

Bea-Mascato B[1,2,3], Neira-Goyanes E[1], Valverde-Pérez D[1,2,3]

1. Departamento de Bioquímica, Genética e Inmunología, Facultad de Biología, Universidad de Vigo, Vigo, Pontevedra, España. 2. Instituto de Investigación Sanitaria Galicia Sur (IIS-Galicia Sur), Pontevedra, España . 3. Centro de Investigaciones Biomédicas (CINBIO), Pontevedra, España

Abstract:

Alström syndrome is a rare disease with a prevalence of 1/1,000,000 per inhabitant. ALMS1 gene has been implicated in this disease, with more than 290 causal mutations, mostly of them located in exons 8, 10 and 16. The majority of these mutations leads to a change of the reading frame that concludes in a premature stop codon, resulting in a truncated protein. The modelling of this disease through KO models becomes in this context an interesting tool to understand the cellular processes that take place in this disease.

We have generated a Knock-out (KO) model in HeLa cell line. We use the CRISPR/Cas9 method with dual system with Homology Direct Repair (HDR). After isolation of homozygous clones, we validated ALMS1 expression by qPCR and Sanger sequencing of the recombinant amplicon comprised between Exon 1 and 3. Then we evaluate mitochondrial activity, proliferation and apoptosis resistance.

We obtained a total inhibition in the expression of the ALMS1 gene. We are performing the characterization but we expected apoptosis resistance, cell cycle elongation and inhibition of mitochondrial activity like other authors report in analysis of patient´s fibroblasts.

The generation of cellular models using CRIPR / Cas9 for the Alström syndrome is considered as a simple and easy to implement tool that would broaden the knowledge of the molecular basis of this disease.

Poster Communication (PC21)

Estimating molecular adaptation in protein-coding sequences accounting for heterogeneous codon frequencies among sites

Del Amparo R, Vicens A, Arenas M.

University of Vigo, Vigo, Spain.

Abstract:

Quantifying selection is fundamental to understand the evolution of genetic data. In this concern, a traditional parameter to evaluate selective constraints in protein-coding sequences is the nonsynonymous/synonymous substitution rate ratio (dN/dS). The estimation of this parameter can be biased if some evolutionary processes, such as recombination, are ignored. Here we investigated a new bias in the estimation of dN/dS caused by variable codon frequencies among codon sites. Applying extensive computer simulations we found an overall underestimation of dN/dS when codon frequencies vary along sequences. Indeed, this underestimation increases with the amount of variability of codon frequencies. Interestingly, we also found that if frequencies only vary at first or second codon positions then dN/dS is underestimated, but if they vary at the third codon position then dN/dS is overestimated. We interpret these biases as a consequence of the violation of assumptions made in common methods to estimate dN/dS. In order to avoid this bias, we propose a methodology based on the independent estimation of dN/dS from partitions formed by codon sites with similar codon frequencies and doing so we could partially reduce this bias. We conclude that accounting for heterogeneous codon frequencies along sequences is crucial to obtain more realistic estimates of molecular adaptation through dN/dS.

Promover o desenvolvemento tecnolóxico, a innovación e unha investigación de calidade.
FEDER. Unha maneira de facer Europa. Centro Singular de investigación de Galicia 2016-2019

Poster Communication (PC22)

Mastocarpus stellatus red seaweed as a promising source of healthy compounds

Torres MD, Flórez-Fernández N, Domínguez H

Department of Chemical Engineering, University of Vigo (Campus Ourense), Polytechnical Building, Ourense, Spain

Abstract:

Mastocarpus stellatus is a model red seaweed belonging to the Gigartinales, Rhodophyta. This carrageenophyte alga is geographically distributed in the Atlantic Iberean Peninsula coast (Galicia and north Portugal). These red seaweeds is demanded by its high content and quality of hybrid carrageenans with particular gelling abilities, required as matrices in the formulation of therapeutic agents, although some controversy with pro-inflammatory properties arise. Antitumoral, antiviral, anticoagulant or immunomodulatory activities were also suggested for extracted fractions of other red seaweeds. The main aim of this work is to recover high valuable compounds with healthy features from M. stellatus red seaweed using green extraction technologies.

M. stellatus red seaweed used as raw material was kindly provided by Portomuíños company (A Coruña, Spain). Subcritical water extraction (autohydrolysis) using compressed hot water in a wide range of temperatures (between 70 to 220 ºC) with a fixed solid:liquid ratio of 1:30 (w:w) was employed to isolate high valuable compounds. Then, the hybrid carrageenans were precipitated with ethanol from the autohydrolysis liquid phases and thermo-rheologically analysed. The corresponding bioactive liquid phases were studied by total phenolic content, sulfate content, protein content, antioxidant or antitumoral activities, among others, using standard methods.

The subcritical water extraction temperature was critically relevant in the molecular weight distribution of the isolated biopolymers, and consequently in the total phenolics and protein content, as well as on the antioxidant and antitumoral activity of the extracts. The obtained results pointed out that subcritical water extraction could be an adequate technology to obtain bioactive and gelling compounds from this model red seaweed, with promising antioxidant, mechanical and biological properties for food, pharmaceutical, cosmetic or even food applications.

Promover o desenvolvemento tecnolóxico, a innovación e unha investigación de calidade.
FEDER. Unha maneira de facer Europa. Centro Singular de investigación de Galicia 2016-2019

Poster Communication (PC23)

Validation of a microfluidic device for the isolation and characterization of circulating tumor cells towards cancer progression monitoring in metastatic breast cancer

Lopes C[1,2], Piairo P[1,3], Corredeira P[3], Costa L[3], Diéguez L[1]

1. Medical Devices research group, Department of Life Sciences, INL- International Iberian Nanotechnology Laboratory, Braga, Portugal. 2. Department of Physics and Department of Biology, University of Minho, Campus de Gualtar, Braga, Portugal. 3. Instituto de Medicina Molecular – João Lobo Antunes, Faculdade de Medicina, Universidade de Lisboa, Lisboa, Portugal

Abstract:

Breast cancer is the second most common cancer worldwide and a leading cause of cancer-related mortality in women. Metastasis is the underlying cause of cancer-related mortality. To improve clinical diagnostic and therapeutic decisions, it's necessary to develop new blood-based biomarker detection strategies. Circulating tumor cells (CTCs) that escape the primary tumor into the bloodstream, exhibit great metastatic potential. CTCs in body fluids represent a real-time snapshot of the tumor burden and offer unique opportunity for minimally invasive sampling in cancer patients. A head to head comparison of a microfluidic device (RUBYchip®, PCT/EP2016/078406) for CTC capture based on cell size and deformability, against the only FDA-approved technology, CellSearch®, was performed. Whole blood samples from metastatic breast cancer patients were collected at baseline (before treatment) and at follow-up (~12 weeks of treatment).
To optimize the performance of the RUBYchip®, breast cancer cell lines, MCF-7, MDA-MB 435 and SKBR3 were used to spike 7.5mL healthy whole blood samples. Different flow rates were tested to assess the isolation efficiency. Double amount of blood specimens were collected in order to test both technologies in parallel. CellSearch® analysis was performed in the Liquid biopsy analysis unit of Santiago (IDIS). Further identification and phenotypical analysis of CTCs was achieved by immunostaining with antibodies against cytokeratin, HER2 and CD45. Results can elucidate and identify CTC subpopulations which could be differentially associated with patients' clinical outcome. Comparing these technologies allows to understand the prognostic value of this sensitive and standardized approach for CTC isolation and phenotypic characterization in breast cancer patients.
Implementation of CTCs in the clinical management of metastatic breast cancer is expected to assist in the monitoring of disease progression, improve patient stratification and prognosis.

Promover o desenvolvemento tecnolóxico, a innovación e unha investigación de calidade.
FEDER. Unha maneira de facer Europa. Centro Singular de investigación de Galicia 2016-2019

Poster Communication (PC24)

3D microsystem with integrated microelectrodes for monitoring cellular metabolic activity

Conceição P, Kant K, Dieguez, L.

Universidade Nova de Lisboa, Campus da Caparica, Caparica, Portugal; International Iberian Nanotechnology Laboratory (INL), Braga, Portugal

Abstract:

The development of 3D tumor models offers several advantages over the 2D cell culture systems such as: free cell growth, spatial structure and organization, which mimics the native extracellular matrix (ECM). These system enables the testing for personalized therapy in drug testing and tissue growth. Standard 2D cell cultures approach are not a real imitator the multicellular organization of solid tumor tissues and usually limited with important contribution of ECM. The microscale sensing under the cell culture environment is very crucial for the development of successful 3D model system. Herein, we fabricated a low-cost microfluidic micro-reactor with the objective of a functional 3D cell culture model system with integrated electrochemical sensing of cell culture microenvironment. The system is based on micro-fabricated device containing a 3D scaffold (Gellun gum hydrogel) for supporting cell culture inside the device. The sensing micro-reactor is fabricated in Poly-methyl methacrylate material, suitable for optical property and low cost. The micro-device is combined with an optical window for inspection of the cells during measurement. The design of the device allowing cell growth with continuous supply of the fresh cell media. A custom developed inlet and outlet system works within an incubator. A cyclic voltammetry is applied to monitor cellular metabolic activity, cellular acidification was accessed with potentiometric pH sensors using gold and platinum electrodes. We observe cell viability for 0-15 day, when breast cancer (MCF-7) cells are cultured in dynamic condition on scaffold. The pH was monitored over the time and changes in the pH were observed inside the micro-reactor over the time, the acidification of cellular microenvironment is observed due to change in pH from 7.4 to 5.8, which reflects the cellular metabolic activity. This system provides the foundation for electrochemical monitoring systems in 3D cell culture diagnostics.

Promover o desenvolvemento tecnolóxico, a innovación e unha investigación de calidade.
FEDER. Unha maneira de facer Europa. Centro Singular de investigación de Galicia 2016-2019

Poster Communication (PC25)

Nanoparticles as a drug delivery system using fucoidan extract

Flórez-Fernández N[1,2], Torres MD[2], Domínguez H[2], Grenha A[1]

1. Centre for Marine Sciences, Faculty of Sciences and Technology, University of Algarve, Faro, Portugal. 2. Departament of Chemical Engineering, University of Vigo (Campus Ourense), Polytechnical Building, Ourense, Spain.

Abstract:

Marine origin polysaccharides have a great potential in several fields due to their features and biological properties. Chemical composition, environmental factors and location, among others, have a key role in their biological activities. Natural compounds extracted from marine organisms have been studied in the last decades. Macroalgae are, in this context, important sources of bioactive compounds. Fucoidan (FUC) obtained from brown seaweeds is a sulfated polysaccharide formed mainly by fucose and sulfate groups. Several biological activities such as antioxidant, anti-inflammatory, antitumoral and antiobesity, have been associated with sulfate groups position. Chitosan (CS) is a natural polymer, obtained from the exoskeleton of crustaceans and formed by N-acetylglucosamine and D-glucosamine units. This biopolymer has been proposed in several biomedical applications. Its positive charge provides a role in the field of drug delivery, as mucoadhesion capacity. Nanoparticles and microparticles are popular drug delivery system, frequently composed by the referred materials.

The aim of this work was to evaluate the conditions of formation of FUC/CS nanoparticles by polyelectrolyte complexation of fucoidan extracted by eco-friendly technology and chitosan. Laminaria ochroleuca was the edible brown seaweed selected to extract fucoidan. Subcritical water extraction was proposed to extract high value compounds using water as a solvent (solid:liquid ratio 1:30). The temperature was previously optimised to obtain the sulfated polysaccharide (160 °C) and fucose content, sulfate content and the antioxidant and antitumoral activities were determined. FUC/CS nanoparticles were produced, varying the polymeric mass ratios. After preparation, nanoparticles were characterised regarding size, polydispersity index and zeta potential. Laminaria ochroleuca was demonstrated to provide fucoidan with suitable characteristics to be complexed with chitosan to form nanosystems.

Promover o desenvolvemento tecnolóxico, a innovación e unha investigación de calidade.
FEDER. Unha maneira de facer Europa. Centro Singular de investigación de Galicia 2016-2019

Poster Communication (PC26)

Shotgun proteomics and schizophrenia: in the search of protein biomarkers in blood

Pardo-Piñón M[6], Mateos Martín J[2], Penedo Fernández-Bujarrabal MA[3], Rivera-Baltanás T[4, 5], Rodrigues-Amorim D[6], Vallejo-Curto MC[6], Martínez Reglero C[7], Balboa Beltrán E[1], Barreiro Villar C[1], Fernández Palleiro P[1], Val Varela V[1], Patiño Álvarez LC[1], Spuch C[1], Olivares Díez JM[1], Carrera Mouriño M[1], Agís-Balboa RC[1]

1. Translational Neuroscience Group, Galicia Sur Health Research Institute -IISGS, Complexo Hospitalario Universitario de Vigo -CHUVI (Álvaro Cunqueiro Hospital). SERGAS-UVIGO, CIBERSAM. Vigo, Pontevedra, Spain. 2. Food Technology, Marine Research Institute (IIM-CSIC). Vigo, Pontevedra, Spain. 3. Nursing Service, CHUVI (Álvaro Cunqueiro Hospital). Vigo, Pontevedra, Spain. 4. Methodology and Statistics Unit, Galicia Sur Health Research Institute -IISGS, CHUVI (Álvaro Cunqueiro Hospital), SERGAS-UVIGO. Vigo, Pontevedra, Spain. 5. Rare Diseases and Pediatric Medicine Research Group, Galicia Sur Health Research Institute -IISGS, CHUVI (Álvaro Cunqueiro Hospital), SERGAS-UVIGO. Vigo, Pontevedra, Spain. 6. IIS Galicia Sur Biobank, Galicia Sur Health Research Institute -IISGS, CHUVI (Álvaro Cunqueiro Hospital), SERGAS-UVIGO. Vigo, Pontevedra, Spain. 7. Virology and Pathogenesis Group, Galicia Sur Health Research Institute - IISGS, CHUVI (Álvaro Cunqueiro Hospital). SERGAS-UVIGO. Vigo, Pontevedra, Spain

Abstract:

Schizophrenia (SZ) is defined as a highly complex multifactorial neuropsychiatric disorder whose pathogenicity is the result of the sum of genetic and environmental risk factors. Currently, its diagnosis is made through clinical interviews and mental evaluations, which have a high reliability but that lack the support of biological tests that corroborate them. Therefore, the search for biomarkers is extremely important when the ultimate goal is to obtain a correct diagnosis, prognosis and/or theragnosis of the disease. In recent years, proteomics has contributed greatly to the identification of new protein biomarkers that identify this pathology. Within the set of techniques that they cover, the proteomic shotgun allows the identification of proteins from a peptide mixture, product of the enzymatic digestion of all the proteins present in a sample. For this, one of the methodologies used is liquid chromatography coupled to mass spectrometry in the system (LC-MS/MS), which allows the separation and subsequent analysis of all the peptides present in the sample. The objective of this study is the search for peripheral protein biomarkers present in the blood through the LC-MS/MS technology, which through a minimally invasive procedure such as a routine blood test, provide information in the clinic about this psychopathology. To do this, we will analyze the proteome of peripheral mononuclear cells - PBMCs (lymphocytes and monocytes) extracted from the blood samples of patients with SZ before and after treatment, as well as the individuals taken as control.

Promover o desenvolvemento tecnolóxico, a innovación e unha investigación de calidade.
FEDER. Unha maneira de facer Europa. Centro Singular de investigación de Galicia 2016-2019

Poster Communication (PC27)

Metallic implants with higher concentrations of Manganese than Titanium and Cobalt could reduce Infection rates in Orthopaedic Surgery

Aveledo R[1], Aveledo A[1], Lago N[2], M Mato M[1], Legido JL[1]

1. Department of Applied Physics, University of Vigo, Vigo, Spain. 2. Pharmacy Service, Alvaro Cunqueiro Hospital, Vigo, Spain.

Abstract:

Infections of joint prosthesis are the most common and feared complications after joint replacements. By far it is the complication that causes more morbidity in these patients, with high costs to national health budget. These implants are manufactured using different metallic alloys. Titanium, cobalt and manganese are metals widely used in these materials, with the latter being used with significantly lower concentrations. In this thermodynamic study, one of the most common bacteria of hospital infections was exposed to solutions prepared with these three metals.
Suspensions of 106 CFU/ml of Pseudomona aeruginosa were mixed with different concentrations of manganese acetate, titanium tetrachloride and cobalt acetate.
Liquid soya-casein-digested was used as culture medium and the samples were introduced in a Calvet microcalorimeter. The physiological temperature of the human body was recreated (309.65 K), and the heat output produced by bacteria metabolism during 48 hours was measured.
All solutions showed proportional inhibition of voltage signal in all thermograms. When comparing the three metallic solutions at certain concentrations, the overall voltage peaks were lower in solutions of manganese than solutions with titanium and cobalt.
Solutions with manganese had higher bactericide effect than other solutions of metals commonly used in metallic alloys of orthopaedic implants. The manufacture of metallic alloys used in this field should take into consideration the potential differences that different metals have in bacteria grow, in order to decrease the risk of infection alongside all the serious consequences to these patients and health care cost.

Promover o desenvolvemento tecnolóxico, a innovación e unha investigación de calidade.
FEDER. Unha maneira de facer Europa. Centro Singular de investigación de Galicia 2016-2019

Poster Communication (PC28)

Detection of recent signatures of selection between three strains of Nile tilapia (Oreochromis niloticus) by whole genome sequencing

Cádiz MI[1], López ME[2], Díaz-Domínguez D[3], Cáceres G[1], Yoshida G[1], Yáñez JM[1]

1. Facultad de Ciencias Veterinarias y Pecuarias, Universidad de Chile, Santiago, Chile. 2. Department of Animal Breeding and Genetics, Swedish University of Agricultural Sciences, Uppsala, Sweden. 3. Departamento de Ciencias de la Computación, Universidad de Chile, Santiago, Chile.

Abstract:

Nile tilapia (Oreochromis niloticus) is the second most cultivated group of fish in the world, mainly because of its favorable characteristics for production. Genetic improvement programs in this species began in 1988 to enhance some traits of interest such as growth rate, disease resistance, cold and salinity tolerance. The implementation of these programs, together with the domestication process of Nile tilapia may have modified the genome through selective pressure, leaving signals that can be detected at the molecular level. In this work, signatures of selection were predicted from genome-wide SNP data by using two complementary methods which are based in the extended haplotype homozygosity (EHH). Whole-genome sequencing of 326 individuals from three strains (A, B and C) of cultured tilapia of two countries (Brazil and Costa Rica) was carried out using Illumina HiSeq 2500 technology. After applying well established SNP-calling and quality-control pipelines, a total of ~1.3M high-quality SNPs were inferred and used as input for the Integrated Haplotype Score (|iHS|) and standardized log-ratio of integrated EHH between pairs of populations (Rsb) methods. Mediated both methods within each strain was possible to detect 16, 174 and 96 genes that could be candidates for selection in A, B, and C strain, respectively. Finally, the enrichment analysis results of all candidates genes to selection were associated with terms (GO and KO) that are important for commercial purposes, such as growth, reproduction, immune system, and behavior. These candidate genes represent putative genomic landmarks that could contain functions of biological and commercial interest.

Promover o desenvolvemento tecnolóxico, a innovación e unha investigación de calidade.
FEDER. Unha maneira de facer Europa. Centro Singular de investigación de Galicia 2016-2019

Poster Communication (PC29)

Beneficial uses of tea branches remains

Sanz V, Domínguez H, Torres MD

Department of Chemical Engineering, University of Vigo (Campus Ourense), Polytechnical Building, Ourense, Spain.

Abstract:

Camellia sinensis L. (green tea) became more and more popular due to its healthy effects such as antioxidant, anticancer, anti-inflammatory, immunomodulatory, hypoglycemic or chemo-preventive activities. Once a year, tea bushes requires to be pruned and their branches and leaves are discarded as a waste. New technologies could allow converting these waste products into valuable raw materials. The main aim of this project is to study new healthy properties of tea from pruning debris using green extraction technologies.

Orballo (A Coruña), a small company located in the Iberian Peninsula, which is pioneer in the cultivation of ecological tea (non-agricultural chemicals production) in Europe, obtaining a great success in its production, kindly provided the by-products. Two environmentally friendly techniques like autohydrolysis and microwave aqueous extraction, were used to recover bioactive compounds from the provided tea branches. In both cases, the processing conditions included temperatures between 140 ºC and 220 ºC and a fixed solid:liquid ratio (1:15). Liquid extracts were analysed for total phenolic, oligosaccharides and protein content, as well as for antioxidant and antitumoral activities. Results indicated that both employed technologies could be attractive alternatives to recover bioactive compounds from tea branches wastes. The temperature exhibited a relevant effect on the total phenolic content and antioxidant capacity, being those samples processed at the highest temperature (220 ºC) those that provided the highest bioactive compounds recovery. The obtained extracts could be suitable agents for food, medicine or pharmaceutical industry and even cosmetic applications. It should be remarked that the development of new products and processes that improve the quality of life, trough green chemistry technology minimizing the use of material, reducing energy and maximizing efficiency is a growing modern trend feasible for these underutilized biomass.

Promover o desenvolvemento tecnolóxico, a innovación e unha investigación de calidade.
FEDER. Unha maneira de facer Europa. Centro Singular de investigación de Galicia 2016-2019

Poster Communication (PC30)

Improving cancer diagnosis and prognosis by identifying tumours with high rates of somatic retrotransposition

Brea-Iglesias J, Rodriguez-Martin B, Juaneda-Magdalena L, MC Tubio J, Martínez-Fernández M

Genomes and Disease Lab. CiMUS (Center for Research in Molecular Medicine and Chronic Diseases). University of Santiago de Compostela (Spain)

Abstract:

The human genome is largely composed of various types of repetitive sequences, including LINE1 (Long Interspersed Nuclear Element 1), which can migrate from their position to other regions, changing the normal genome structure in the places where they integrate. Most LINE 1 are silenced by epigenetic mechanisms under normal conditions. However, in cancer they become activate and mobilize along the genome uncontrollably. Within the PanCancer Analysis of Whole Genomes project, our research group has identified 124 LINE1 elements active in cancer genomes (Tubio et al., 2014, Rodríguez-Martin et al., 2019). Our data supported that mobilization of LINE1 is especially active in oesophageal adenocarcinoma, head and neck carcinoma, lung carcinoma and colorectal adenocarcinoma (Rodríguez-Martin et al., 2019). Thus, mobilization of LINE1 elements can work as oncogenes, by producing new mutations and rearrangements with a driver role in cancer. Unfortunately, its activation is not currently evaluated at cancer diagnosis, missing an important mechanism favouring tumor progression.

This study focuses on the development and optimisation of a molecular test to allow the estimation of LINE1 activation in tumour samples. This technique is based on Agilent SureSelectXT2 for Illumina Multiplexed Sequencing Protocol, targeting the 124 LINE1 active elements in cancer. To evaluate this molecular test, we used cancer cell lines whose retrotransposition rate was previously determined. Then, the applicability of the test has been assessed in frozen tumor samples and Formalin-Fixed Paraffin Embedded tumor tissues from head and neck, colorectal and lung cancer patients, confirming their applicability.

The potential implementation of this technique in clinical practice will result in a more precise cancer diagnosis, which could lead to the selection of a more personalized treatment and to increase a preventive close follow-up in those patients with a high rate of somatic retrotransposition.

Promover o desenvolvemento tecnolóxico, a innovación e unha investigación de calidade.
FEDER. Unha maneira de facer Europa. Centro Singular de investigación de Galicia 2016-2019

Poster Communication (PC31)

Influence of the last glacial period and admixture on genetic gradients of current Asians

Branco C[1,2], Arenas M[1,2]

1. Department of Biochemistry, Genetics and Immunology, University of Vigo, Vigo, Spain 2. Biomedical Research Center (CINBIO), University of Vigo, Vigo, Spain.

Abstract:

Patterns of genetic variation in diverse species, including modern humans, may be associated with the distribution of some diseases throughout the world. Cavalli-Sforza and coauthors (1993) observed a genetic gradient in Asia with an east-west orientation, which they believed to be a consequence of past expansions; however, little was explored concerning the environmental and population genetic processes causing this gradient. Here we evaluated the influence of range contractions caused by the last glacial period and admixture between Paleolithic and Neolithic populations on genetic gradients of current Asians. We applied spatially explicit simulations to mimic those evolutionary scenarios and we estimated the corresponding genetic gradients with principal component analyses. Then, we analyzed the genetic gradients to identify genetic processes that might have occurred during the colonization of the continent and to compare the simulated genetic gradients with the real gradients to identify the best fitting evolutionary scenario. Our results showed that (1) Paleolithic populations present the real east-west gradient only if the last glacial period is considered and, under this situation, the genetic gradient was probably caused by allele surfing; (2) Scenarios with admixture between Paleolithic and Neolithic populations presented the real east-west gradient only if two origins of Neolithic expansion are considered. This gradient could be explained by admixture of genetic sectors caused by the Neolithic expansions. Additionally, we found genetic isolation in the Arabian Peninsula and in Japan, probably caused by geographic isolation. Altogether we conclude that the last glacial period promoted the real genetic gradient observed in Asia although other factors (especially the Neolithic expansion from two origins) could also favor the gradient.

Promover o desenvolvemento tecnolóxico, a innovación e unha investigación de calidade.
FEDER. Unha maneira de facer Europa. Centro Singular de investigación de Galicia 2016-2019

Poster Communication (PC32)

Detection of resistance mutations in NSCLC by NGS using liquid biopsy

Oitabén A[1], Prado-López S[1,2], Tomás L[1], Huidobro G[3], Posada D[1,2]

1. Department of Biochemistry, Genetics and Immunology, University of Vigo, Spain. Biomedical Research Center (CINBIO), University of Vigo, Spain. 2. Galicia Sur Health Research Institute, Vigo, Spain. 3. FEA Oncoloxía Médica, Hospital Álvaro Cunqueiro, Vigo, Spain.

Abstract:

Lung cancer is the most common cancer type worldwide (AECC) and it was the main death cause in Spain in 2018 (SEOM). More than 29,000 new cases are detected every year in our country and each one has specific characteristics, mainly due to genomic heterogeneity. The latter varies responses to drugs (Dzobo et al., 2018), making necessary the development of precise and non-invasive tools, to monitor tumor evolution and to detect drug resistance.
We are currently studying studied advanced non-small cell lung cancer (NSCLC) (stage IV) patients, with activating mutations in EGFR, treated with EGFR-TKIs. We isolated cell-free DNA (cfDNA) from plasma (QIAamp Circulating Nucleic Acid Kit, Qiagen) and total DNA from saliva (QIAamp DNA Blood Mini kit, Qiagen) at different time points, to assess genomic changes in the tumor and the evolution of acquired resistance mechanisms. We are using a targeted panel specific for lung cancer, including 72 different genes (QIAseq Human Lung Cancer Panel, Qiagen). Next-generation sequencing will be carried out with the IonTorrent platform at high depth (2500x), in order to detect tumor mutations at very low frequencies.

References:
Asociación Española Contra el Cáncer (AECC). 'Todo sobre el cáncer. Cáncer de pulmón' [Online acces: 11/05/2019] https://www.aecc.es/es/todo-sobre-cancer/tipos-cancer/cancer-pulmon.
Dzobo, K. et al. (2018) 'Not Everyone Fits the Mold: Intratumor and Intertumor Heterogeneity and Innovative Cancer Drug Design and Development', OMICS: A Journal of Integrative Biology, 22(1), pp. 17–34. doi: 10.1089/omi.2017.0174.
Sociedad Española de Oncología Médica (SEOM). 'Las cifras del cáncer en España 2019'. [online acces: 10/05/2019] https://seom.org/dmcancer/wp-content/uploads/2019/Informe-SEOM-cifras-cancer-2019.pdf

Promover o desenvolvemento tecnolóxico, a innovación e unha investigación de calidade.
FEDER. Unha maneira de facer Europa. Centro Singular de investigación de Galicia 2016-2019

Poster Communication (PC33)

BL-1249 induces a clear hyperpolarization and a TREK-like current in mouse sympathetic neurons

Campos-Ríos A, Herrera-Pérez S, Rueda-Ruzafa L, Rodríguez-Piñeiro A, Lamas JA

University of Vigo, Vigo, Spain.

Abstract:

The TREK channels (TREK-1, TREK-2 and TRAAK) are members of two-pore-domain potassium (K2P) channel family, which contribute to the background potassium conductance in many cell types. TREK channels have been implicated in a multitude of pathological conditions such as chronic pain, ischemia, cancer and depression. For this reason, the pharmacological modulation of the TREK channels is of great interest. On this matter BL-1249, a compound derived from the family of non-steroidal anti-inflammatories, has been shown to be a good activator of TREK channels (*Fernández-Fernández et al., PLoS One 13(6):e0199282, 2018*). We have used the perforated patch-clamp technique (whole-cell) to analyse the effect of BL-1249 on sympathetic neurons of the mouse Superior Cervical Ganglion (SCG).
The application of BL-1249 induces a clear hyperpolarization and reduces the firing, even if the hyperpolarizing effect of the drug is counteracted by current injection. We have also confirmed that this hyperpolarization is caused, at least in part, by the activation of potassium channels. Pharmacological experiments, using blockers of other ion channels, demonstrated that the current induced by BL-1249 is conducted through TREK channels in SCG neurons.

Key words: BL-1249, TREK-1, Superior Cervical Ganglion
Acknowledgements: Funds from Spanish Government (MINECO, BFU2014-58999-P), Galician Government (GPC2015/022) and European Regional Development Fund (FP7-316265-BIOCAPS). Partially supported with FEDER Funds.

Poster Communication (PC34)

Biological properties of gold and silver nanparticles from green synthesis

González-Ballesteros N[*1], Diego-González L[*2], Simón-Vázquez R[2], Lastra-Valdor M[3], Rodríguez-Argüelles MC[1]

1. Departamento de Química Inorgánica. Centro de Investigaciones Biomédicas (CINBIO). Universidade de Vigo, Vigo, Spain. 2. Inmunología. Centro de Investigaciones Biomédicas (CINBIO). Instituto de Investigación Sanitaria Galicia Sur (IIS-GS). Universidade de Vigo, Vigo, Spain. 3. Estación de Ciencias Marinas de Toralla (ECIMAT), Universidade de Vigo, Vigo, Spain
*Co-first authors

Abstract:

Seaweeds are a great source of bioactive compounds with a wide variety of applications. Recently they have been named as "bionanofactories" due to their ability for the synthesis of metal nanoparticles. The development of green synthesis methods is in potential growth since, among other advantages, they reduce the use of toxic and expensive reagents, thus being eco-friendly and cost-effective[1].
We have selected the brown macroalgae Sacchoriza polyschides (SP) for the green synthesis of gold and silver nanoparticles (Au@SP and Ag@SP). SP is a large, kelp-like macroalgae, broadly distributed in the lower littoral of the North East Atlantic coast. SP extracts have recently been studied due to their hypoglycemic, antiplasmodial, anti-inflammatory and cytotoxic features[2].
Firstly, an aqueous extract of SP was prepared and employed to lead the synthesis of nanoparticles by the reduction of $HAuCl_4$ and $AgNO_3$ as previously reported[3]. The characterization confirmed the synthesis of spherical, polycrystalline nanoparticles with mean diameter of 14±2 nm for Au@SP and 15±3 nm for Ag@SP. In vitro antioxidant activity of SP extract before and after the synthesis was determined by the analysis of the reducing power, total phenolic content and DPPH scavenging activity.
The potential toxicity of SP extract, Au@SP and Ag@SP was evaluated in three human cell lines at different doses. All concentration tested were safe and a proliferative effect was detected at low and intermediate concentrations, mainly with the SP extract. Besides, the samples were able to induce ROS production at the highest doses. The data obtained suggest that Au@SP and Ag@SP could have proliferative properties and mediated by ROS signaling[4].

References:
[1] A.U. Khan et al, Bioprocess Biosyst Eng. 42 (2019) 1-15
[2] S.A. Dahoumane et al, Green Chem. 19 (2017) 552-587
[3] N. González-Ballesteros et al, XXII Encontro Luso-Galego de Química, Bragança (Portugal) 2016
[4] M. Schieber et al, Curr Biol. 24 (2014) 453-462

Promover o desenvolvemento tecnolóxico, a innovación e unha investigación de calidade.
FEDER. Unha maneira de facer Europa. Centro Singular de investigación de Galicia 2016-2019

Poster Communication (PC35)

New researchers of peloids for therapeutic uses

P. Gómez C[1], Mourelle ML[2], M Mato M[2], Tobar JL[2] , F Marcos MD[2], Casás L[3], Legido JL[2]

1. The Biomedical Research Centre (CINBIO), University of Vigo, Vigo, Spain. 2. Department of Applied Physics, University of Vigo, Vigo, Spain. 3. Univ. Pau & Pays Adour, Laboratoire de Thermique, Energétique et Procédés-IPRA, Pau, France.

Abstract:

This paper presents the most recent researches on thermophysical properties of peloids elaborated from mixtures of clays with mineral waters of different mineralization, for their therapeutic application in thermal spas, and thalassotherapy centers. The properties studied have been density, thermal conductivity, specific heat and thermal diffusivity.
The density of the liquid phase has been determined by means of an Anton Paar DMA 4500 densimeter while the density of the mixtures has been carried out by means of a pycnometric method. A Decagon KD2 Pro conductivitymeter was used to determine the thermal conductivity. The specific heat was determined using a CALVET microcalorimeter, while the thermal diffusivity was calculated from the density, specific heat and thermal conductivity data obtained. The properties have been determined at different temperatures and at atmospheric pressure.
In this work an analysis of the behaviour of the properties depending of the concentration and the temperature is made, looking for those mixtures that present better properties for its therapeutic application.
Acknowledgments. The authors are grateful to Maria Perfecta Salgado González for her collaboration with the experimental measurements. We are also thankful for the financial support provided by the projects ED431C 2016-034 “Axudas a Grupos de Referencia Competitiva” funded by Xunta de Galicia and ED431G/02 “Centro de Investigacións Biomédicas” (CINBIO) co-financed with FEDER funds.

Poster Communication (PC36)

Bootstrap-based methods for testing linear combinations of proportions. Application to real data of the Flemish Cap Bank

Álvarez Hernández M[1], Roca-Pardiñas J[2], González-Troncoso D[3]

1. Centro Universitario de la Defensa, ENM, Univesity of Vigo, Spain. 2. University of Vigo, Spain. 3. Centro Oceanográfico de Vigo (IEO), Spain.

Abstract:

In recent years, inferences about a linear combination of proportions have aroused a great amount of interest, especially in applied research. By this way, in the present work studies were realized about the status of a fishing species in which, because of the type of sampling that is performed, the objective value is treated as a linear combination of independent proportions.
We introduce a bootstrap method which is a competitive procedure and a good alternative vs methods proposed in literature (mostly asymptotic methods). The bootstrap procedure additionally could allow to expand the findings in order to compare several linear combinations of independent proportions.
The methodology will be applied to real data of the cod in the Flemish Cap Bank (NAFO Division 3M). Biologists have assumed that the growth for adult males and females is the same in this stock, so length distributions are not sorted by sex. We must determine if the separation of the catch into sex is adequate and, in this case, if the differences between the proportion of sex are maintained over the years.

Promover o desenvolvemento tecnolóxico, a innovación e unha investigación de calidade.
FEDER. Unha maneira de facer Europa. Centro Singular de investigación de Galicia 2016-2019

Poster Communication (PC37)

Plasmonic nanoparticles@MOF hybrids as SERS-tags for biodetection

De Marchi Lourenço S, Bodelón G, Vázquez-Iglesias L, Pérez-Juste J, Pastoriza-Santos I

Department of Physical Chemistry and Biomedical Research Centre (CINBIO), Universidad de Vigo, Vigo, Spain.

Abstract:

Metal organic frameworks (MOFs) are a new class of materials with interesting properties, such as large internal surface area, tunable crystal porosity and high chemical versatility. Zeolitic imizadolate frameworks (ZIFs) are a type of MOF composed by imidazolate linkers connected by transition metals cations. In an attempt to fabricate new hybrid materials with novel or enhanced behavior, the combination of ZIFs and plasmonic metal nanoparticles appears as a powerful alternative. Plasmonic nanostructures are characterized by their outstanding optical properties based on localized surface plasmon resonance (LSPR). Once excited by light, a strong and localized electromagnetic field at the nanoparticle surface is generated. It can affect molecules placed nearby the metal surface, thus in the case of Raman active molecules a significant increase in their Raman signal is produced. It forms the basis of the Surface Enhanced Raman Scattering (SERS) spectroscopy, a powerful analytical technique which allows the ultrasensible detection of molecules.
In this study we combine a plasmonic core (Au@Ag core-shell nanorod) and a ZIF-8 shell (Zn[2-methylimizadole]2) to fabricate MOF-based SERS tags. To this aim, Au@Ag@ZIF-8 nanoparticles were encoded with Raman active molecules and functionalized with biomolecules capable of specific targeting cell receptors. A robust and oriented immobilization of poly-histidine-tagged protein G on the ZIF-8 surface were achieved through the direct coordination of unsaturated Zn2+ and imidazole moiety of histidine molecules. The capability of cell imaging using the plasmonic@MOF hybrids was demonstrated through their conjugation with antibodies against the epidermal growth factor receptor (EGFR) and cell surface adhesion receptor CD44, which allowed us to discriminate A431 (EGFR+, CD44+) from 3T3 2.2 (EGFR−, CD44+) cells by means of SERS.

Promover o desenvolvemento tecnolóxico, a innovación e unha investigación de calidade.
FEDER. Unha maneira de facer Europa. Centro Singular de investigación de Galicia 2016-2019

Poster Communication (PC38)

Identification of a putative metastasis-related gene in a preclinical breast cancer model

Martínez Pena I, Hurtado Blanco P, Pereira Veiga T, Abuín Redondo C, Costa Nogueira C, López López R, Piñeiro Cid R

Roche-CHUS Joint Unit. Translational Medical Oncology Group, Oncomet. Health Research Institute of Santiago de Compostela, Spain.

Abstract:

Breast cancer (BC) is the most frequent tumour in women. Approximately, 90% of cancer-related deaths are due to the development of metastasis. Metastasis is a multi-step process by which new tumour lesions are generated far from the primary tumour (PT). Circulating Tumour Cells (CTCs) are those tumour cells released into the bloodstream. CTCs have the potential to seed metastasis and their presence in peripheral blood of metastatic BC patients is associated with a worse disease outcome.

Our objective is to understand the mechanisms of BC metastasis for the identification of markers of potential relevance for the biology of CTCs.

We have generated an orthotopic BC murine model using a human triple-negative BC cell line. PT and lung metastasis were removed to obtain paired cell lines, which were used to perform gene expression analysis by real time PCR and RNAseq. Additionally, blood samples were collected and CTCs isolated by ParsortixTM technology for downstream gene expression assays.

Transcriptome assays have shown significant differences in eight genes between paired cell lines. One of these genes was found to be up-regulated in the metastases-derived cell lines and could have therapeutic implications.

We were able to isolate single CTCs and CTC-clusters from murine xenograft blood samples. We are currently evaluating the expression of the candidate gene in CTCs isolated from peripheral blood samples of metastatic BC patients and in CTCs isolated from the blood of our murine model. We are now starting to validate these data at a protein level and assessing the therapeutic implications of the putative metastasis-related gene.

Our murine BC model allowed us to identify a putative metastasis-related gene with therapeutic potential and to study BC metastasis at different levels, such as PT, CTCs and metastasis. Further work will be directed to assess the function of this gene regarding tumour spread.

Promover o desenvolvemento tecnolóxico, a innovación e unha investigación de calidade.
FEDER. Unha maneira de facer Europa. Centro Singular de investigación de Galicia 2016-2019

Poster Communication (PC39)

Disseminated CTC-clusters have greater survival in zebrafish xenograft compared with single CTCs

Hurtado Blanco P[1], Martínez Pena I[1], Abuín Redondo C[1], Sánchez Piñón L[2], López López R[1,3], Piñeiro Cid R[1]

1. Roche-CHUS Joint Unit. Translational Medical Oncology Group, Oncomet. Health Research Institute of Santiago de Compostela (IDIS). 2. Genetics Department, Veterinary Faculty, Universidad de Santiago de Compostela. 3. Translational Medical Oncology Group, Oncomet. University Hospital of Santiago de Compostela (CHUS).

Abstract:

Most of deaths associated with cancer are caused by metastasis, in which tumour cells can enter the blood stream as circulating tumour cells (CTCs) and establish new tumour foci. These CTCs can travel as single cells or in groups called CTC-clusters, which are believed to have a greater metastatic capacity. Our objective is to understand the biology of CTC-clusters and their contribution to metastasis, through their functional characterization. We use an in vitro model of CTCs and CTC-clusters generated from a triple negative breast cancer cell line. Both populations are injected in the circulation of zebrafish embryos, where we can track the fluorescent labelled cells to study tumour spreading. Afterwards, zebrafish bodies are disaggregated and the disseminated tumor cells are isolated by micromanipulation and subjected to downstream analysis. Zebrafish experiments shows that both CTCs and CTC-clusters can survive in the zebrafish circulation and efficiently disseminate and seed metastases. We have observed a greater mobility in the single cell population, while clustered cells seem to better survive once nested in the zebrafish tail. Our data shows the ability of circulating tumour cells to disseminate and form metastasis into the zebrafish embryo. We have observed preliminary differences in dissemination and survival between CTCs and CTC-clusters in our xenograft model, and we are able to isolate migrated tumour cells in order to perform a wide range of analysis. Therefore, our CTC-cluster model is a promising model to study the biology of these groups of cells and their contribution to metastasis.

Promover o desenvolvemento tecnolóxico, a innovación e unha investigación de calidade.
FEDER. Unha maneira de facer Europa. Centro Singular de investigación de Galicia 2016-2019

Poster Communication (PC40)

Crude fucoidan fractions with cytotoxic properties

Flórez-Fernández N[1,2], Torres MD[2], Domínguez H[2]

1. University of Algarve, Portugal. 2. University of Vigo, Spain.

Abstract:

Fucoidans are heteropolysaccharides composed of fucose, uronic acids, galactose, xylose, mannose, arabinose, glucose and sulfate groups, and are exclusively found in brown seaweeds. The low molecular weight fucoidan fractions show improved bioavailability and a broad range of potential health benefits without toxic effects. The chemical composition and structure of fucoidans is taxonomically dependent and is influenced by seasonal and environmental conditions, as well as by collecting and the extraction/purification techniques. Fucoidan is attracting increasing interest as a new anti-cancer compound, showing promising activity against different tumoral cell types without minimal or no side effects on normal cells and also presents synergistic activity with other chemotherapeutic agents. However, the effects of the fucoidan composition and structural features on the antitumoral activity are not clear.

Fucoidans are usually extracted from brown algae in multistep processes using hot, dilute acid, or high temperature water. The increasing health and environmental consciousness has led to the development of eco-friendly, innovative technologies with reduced energy consumption, emissions and costs as well as to increase safety and products quality. Hydrothermal treatments with subcritical water proved as an efficient technology for the simultaneous extraction and depolymerization of fucoidan fractions from brown seaweeds.

The objective of this work is to explore the cytotoxic properties of crude fucoidans extracted from Galician brown seaweeds using hydrothermal treatments with pressurized hot water under subcritical conditions. The concentration dependent cytotoxic action of crude fucoidan has been observed and the EC50 values were in the range reported for other natural products. The influence of composition (monomeric constituents, sulfation degree), molecular weight and the presence of other components, such as phlorotannins, has also been assessed.

Promover o desenvolvemento tecnolóxico, a innovación e unha investigación de calidade.
FEDER. Unha maneira de facer Europa. Centro Singular de investigación de Galicia 2016-2019

Poster Communication (PC41)

Novel Fe3O4@Al(OH)3 nanostructures for dual modal PET/MRI imaging

González-Gómez MA, Yáñez-Vilar S, Piñeiro Y, Rivas J

University of Santiago de Compostela, Santiago de Compostela, Spain.

Abstract:

Molecular imaging methods such as magnetic resonance imaging (MRI) and positron emission tomography (PET) have their own limitations in terms of spatial resolution, sensitivity, and depth of signal penetration that might be overcome by the combination of two or more such modalities. Therefore, dual imaging are currently being explored to enhance the quality and specificity of imaging methods in order to achieve proper visualization of organs or to better localize the accumulation of a diagnostic or therapeutic agent.

The development of magnetic nanoparticles has been serving as a platform for MRI contrast agents due to their tunable properties such as magnetism, size, and facile conjugation with biologically functional units. In addition, their surface functionalization provides them with the ability to carry a wide range of imaging moieties, such as radioisotopes (18F, 89Zr), which makes nanoparticles useful in multi-modal imaging systems.

In this study it was developed a novel route to obtain a biocompatible superparamagnetic magnetite with excellent magnetic properties coated with aluminium hydroxide that provides these promising nanostructures with high affinity with fluoride and zirconia anions for use in contrast abilities for MRI and PET imaging.

Promover o desenvolvemento tecnolóxico, a innovación e unha investigación de calidade.
FEDER. Unha maneira de facer Europa. Centro Singular de investigación de Galicia 2016-2019

Poster Communication (PC42)

Biocompatible matrices for transdermal drug delivery applications

Puig J[1,2], González-Gómez MA[1], Yáñez-Vilar S[1], Piñeiro Y[1], Hoppe CE[2], Rivas J[1]

1. University of Santiago de Compostela, Spain 2. INTEMA (Institute of Materials Science and Technology, CONICET-UNMDP)

Abstract:

Transdermal delivery system offers a non or minimally invasive, painless procedure and can be self-administrated.1,2 Stimulus-responsive systems for on demand release are desirable for the controlled release of active principles over long-term periods.3 PEG-based hydrogels are widely used in a variety of biomedical applications due to their excellent biocompatibility and high hydrophilicity, including matrices for controlled release of biomolecules and scaffolds for regenerative medicine.4 Magnetic responsive hydrogels can be remotely controlled upon exposure to an alternating magnetic field (AMF).5 A magnetic field can deform a hydrogel network containing magnetic nanoparticles and enhances the release of drug molecules.6
In this work, a hydrogel that incorporate the magnetic nanoparticles (MNPs) and the active agent during polymerization is described. An UV-photopolymerized PEG-based hydrogel is used as matrix. Dopamine is used as the model drug. The drug release behavior in response to an AMF is investigated. The possibility of reloading the dopamine by simple infiltration is explored.

References:
1. Kim, B. et al. Touch-actuated transdermal delivery patch for quantitative skin permeation control. Sens. Actuators B Chem. 256, 18–26 (2018).
2. Amjadi, M., Sheykhansari, S., Nelson, B. J. & Sitti, M. Recent Advances in Wearable Transdermal Delivery Systems. Adv. Mater. 30, 1704530 (2018).
3. Teodorescu, F. et al. Photothermally triggered on-demand insulin release from reduced graphene oxide modified hydrogels. J. Controlled Release 246, 164–173 (2017).
4. Lin, C.-C. & Anseth, K. S. PEG Hydrogels for the Controlled Release of Biomolecules in Regenerative Medicine. Pharm. Res. 26, 631–643 (2009).
5. Zhao, X. et al. Active scaffolds for on-demand drug and cell delivery. Proc. Natl. Acad. Sci. 108, 67–72 (2011).
6. Li, J. & Mooney, D. J. Designing hydrogels for controlled drug delivery. Nat. Rev. Mater. 1, (2016).

Promover o desenvolvemento tecnolóxico, a innovación e unha investigación de calidade.
FEDER. Unha maneira de facer Europa. Centro Singular de investigación de Galicia 2016-2019

Poster Communication (PC43)

Multifunctional silica magnetic nanoparticles for magnetic uptake and cell separation applications

Seco-Gudiña R[1,2], González-Gómez MA[1], Yáñez-Vilar S[1], Piñeiro Y[1], de la Fuente M[2], López-López R[2], Rivas J[1]

1. Applied Physics Department, NANOMAG Laboratory, Research Tecnological Institute, Universidade de Santiago de Compostela (USC), Spain. 2.Translational Medical Oncology, Health Research Institute of Santiago (IDIS); Clinical University Hospital of Santiago de Compostela, Spain

Abstract:

Isolation of key cell populations to perform accurate diagnosis, is one of the greatest challenges in biomedicine, especially in oncology . A new approach based on an enhanced cell selection by the use of magnetic nanoparticles (MNPs) is emerging as a new tool in biomedical procedures. Due to the ability of MNPs to be manipulated by an external magnetic field, their uptaking by cells offers unique possibilities like magnetic hyperthermia applications; gene/drug delivery, antibody magnetic detection and selective magnetic separation of tagged cells. In the present work we have performed magnetic cell tagging experiments using HeLa cells (CCL-2 ATCC) and a set of different MNPs (with a fixed concentration of 100µg/mL) , consisting in NdFeB alloy or magnetite cores, coated with different organic and inorganic shells (silica, dopamine, etc.) and functionalized with fluorescent moieties (rhodamine and fluorescein). Two procedures were studied: passive uptaking of NPs by cancer cells and magnetically active uptaking of NPs forced by a permanent magnet. This resulted in the shortening of incubation time from 3/4 hours in the passive case versus 15 minutes in the magnetically driven one. In addition, analyzed with the help of a confocal microscope, it was observed that the magnetically driven procedure ended up in an increased uptake of MNPs by the cells. Moreover, MNPs loaded cells' viability was assessed using cell metabolic activity (MTT), showing that the magnetically driven set presented a reduced cytotoxicity. These results allow us to propose magnetically driven uptake of MNPs by cells as an efficient procedure for cell manipulation with minimum cell stress.

Promover o desenvolvemento tecnolóxico, a innovación e unha investigación de calidade.
FEDER. Unha maneira de facer Europa. Centro Singular de investigación de Galicia 2016-2019

Poster Communication (PC44)

Detection of TiO2 nanoparticles by their photocatalytic activity

Tiryaki E[1,2,3], Pérez-Lorenzo M[1,2,3], Correa-Duarte MA[1,2,3]

1.Department of Physical Chemistry, Center for Biomedical Research (CINBIO). 2.Southern Galicia Institute of Health Research (IISGS). 3.Biomedical Research Networking Center for Mental Health (CIBERSAM), Universidade de Vigo, Vigo, Spain

Abstract:

Titanium dioxide nanoparticles (TiO2 NPs) are one of the most manufactured semiconductors worldwide. This extensive use has given rise to an increasing release of these materials into the environment which may constitute a risk from an ecological point of view. Along these lines, the formation of reactive oxygen species (ROS) as a result of the irradiation of TiO2 NPs by UV light, may harm individual organisms through mechanisms of oxidative stress resulting in various toxicity effects.1 For that reason, the implementation of methods capable of determining the adverse effects of these materials is of utmost importance. In this study, we aim at determining the influence that dosage as well as different morphological features (crystallinity, surface/mass ratio, etc.) of TiO2 NPs exerts on their toxicity in aqueous media. To this end, the photocatalytic degradation of an organic dye in the presence of different TiO2 nanoparticles have been performed.

Promover o desenvolvemento tecnolóxico, a innovación e unha investigación de calidade.
FEDER. Unha maneira de facer Europa. Centro Singular de investigación de Galicia 2016-2019

Poster Communication (PC45)

Multifunctionalized silica magnetic nanoparticles for biomedical applications

García Acevedo P, González Gomez M, Yanez Vilar S, Piñeiro Y, Rivas J

University of Santiago de Compostela, Santiago de Compostela, Spain.

Abstract:

Personalized medicine is one of the challenges of health in developed countries, and requires highly selective and control therapeutic methods to perform diagnoses, therapeutic designs, highly controlled dosages and "in situ monitoring". For this reason, in this study, we synthetized a facile development of magnetite coated with silica magnetic nanoparticles for their applicability as a biocompatible T2 contrast agent for MRI of biological tissues and magnetic hyperthermia applications . In addition, plasmonic functionality is added by linking gold NPs to the magnetic nanoparticles or silica surface in order to provide with photothermal and optical abilities.
Moreover, the gold coating shell offers the advantage of being colloidally stable, biocompatible, provides protection against oxidation and adds chemical versatility to the nanostructure surface, allowing for the conjugation of targeting ligands and drugs by using the well-known gold-thiol chemistry.
These magnetic gold coated nanostructures, allow for a combined photothermal and magnetic hyperthermia response, to kill cancer cells or to serve as biomarkers detectors based on their magnetic and optical response.
Also, a properly functionalization of the silica shell with a fluorescent biomarker, achieves a new dual-marker particle, simultaneously combining fluorescent, optical and magnetic properties in a single entity. These dye-conjugated magnetic nanoparticles are preferred for in vivo applications since quantum dots contain toxic elements such as cadmium or selenium.
In this work, we describe the encapsulation of magnetite NPs with different morphology and magnetic properties and different coatings (silica, gold) synthetized by different methods (co-precipitation, thermal decomposition, and inverse microemulsion) and the physicochemical characterization of their properties.

Promover o desenvolvemento tecnolóxico, a innovación e unha investigación de calidade.
FEDER. Unha maneira de facer Europa. Centro Singular de investigación de Galicia 2016-2019

Poster Communication (PC46)

Synthesis and characterization of superparamagnetic Photonic Crystals

Arnosa-Prieto A[1], Sánchez-Andújar M[1], Castro-García S[1], Rivas J[2]

1. University of A Coruña, Spain. 2. University of Santiago de Compostela, Spain

Abstract:

In the past decade, responsive photonic crystals (PCs) have acquired considerable attention. Their unique magnetic responsiveness provides a wide range of applications in different fields. PCs show advantages in anticounterfeiting and information encryption technologies but they are arising as a new trend in biomedicine: detection of different diseases, drug delivery and tumour screening. Among different types of PCs, colloidal assembled magnetic responsive photonic crystals are the most interesting due to their reversible and tunable response to magnetic fields.

The magnetic responsive photonic crystals prepared in this work, are characterized by their magnetochromism: a reversible and tunable change in their colour in response to the application of a magnetic field.

Synthesized superparamagnetic Fe3O4 colloidal nanocrystal clusters (MCNCs) present a core-shell structure. MCNCs have been prepared using a modified one-pot solvothermal method where anionic polyelectrolyte poly(4styrenesulfonic acid-co-maleic acid) sodium salt (PSSMA) was used as stabilizer to obtain the core. A modified Stöber process was applied for silica coating to complete the core-shell nanostructure.

The synthesized Fe3O4@PSSMA@SiO2 was characterized with transmission electron microscopy (TEM) and dynamic light scattering (DLS). TEM images showed the spherical shape and homogeneous size of the particles. An average diameter of 269 nm was obtained by DLS.

The magnetization curve for Fe3O4@PSSMA reveals a superparamagnetic behaviour with a value for the remnant magnetization of 0.65 emu·g-1 at room temperature.

Promover o desenvolvemento tecnolóxico, a innovación e unha investigación de calidade.
FEDER. Unha maneira de facer Europa. Centro Singular de investigación de Galicia 2016-2019

Poster Communication (PC47)

Viral mimetic assemblies: novel nanostructures for drug delivery

Marín-Caba L[1], Iturrioz-González N[2], Chariou PL[3], Steinmetz NF[3], Fanarraga ML[2], Correa-Duarte MA[1]

1. University of Vigo, Spain . 2. University of Cantabria, Spain. 3. University of California-San Diego, USA

Abstract:

Recently, the design of versatile tools to improve cell targeting and drug delivery in medicine is taking upmost relevance in nanobiotechnology. Biological and inorganic nanocarrier drug delivery systems have their advantages and disadvantages in terms of cell targeting and specificity, cell internalization, efficient payload delivery, and safety profiles(1,2). Herein, we present two different nanostructures that present relevant characteristics as drug delivery systems. Both nanohybrids are composed by mesoporous silica nanoparticles (MSNP), although the organic coating over the silica surface that make them advantageous is different. One of them is covered by multi-walled carbon nanotubes (MWNT), named MWNT@MSNP, while the other nanohybrid is covered with a biological coating, Tobacco Mosaic Virus (TMV) nanoparticles, named TMV@MSNP. MWNT@MSNP are capable of escape from endosomes after 72 h, by mimicking the spike-shape from animal virus(3). On the other hand, TMV@MSNP, a wool ball-like nanostructure, showed a higher cell uptake than bare MSNP due to the TMV biological coating(4). These outstanding features, together with the high loading capacity of MSNP, make them ideal candidates as drug delivery systems.

References:
[1] Gao, J. & Xu, B. Applications of nanomaterials inside cells. Nano Today. 4, 37–51 (2009).
[2] Jutz, G. & Böker, A. Bionanoparticles as functional macromolecular building blocks - A new class of nanomaterials. Polymer. 52, 211–232 (2011).
[3] Iturrioz-Rodríguez, N., González-Domínguez, E., González-Lavado, E., Marín-Caba, L., Vaz, B., Pérez-Lorenzo, M., Correa-Duarte M.A., Fanarraga, M.L. A Biomimetic Escape Strategy for Cytoplasm Invasion by Synthetic Particles. Angew Chem Int Ed Engl. 56(44), 13736-13740 (2017)
[4] Marín-Caba, L., Chariou, P. L., Pesquera-González, C., Correa-Duarte, M. A., Steinmetz, N. F. Tobacco Mosaic Virus functionalized mesoporous silica nanoparticles. Langmuir. 35, 203−211 (2019)

Promover o desenvolvemento tecnolóxico, a innovación e unha investigación de calidade.
FEDER. Unha maneira de facer Europa. Centro Singular de investigación de Galicia 2016-2019

Poster Communication (PC48)

A SERS based 3D hybrid biosensor for lactate detection and monitoring

Abalde-Cela S[1], Rebelo R[2,3], Wu L[1], Barbosa AI[2,3], Rodríguez-Lorenzo L[1], Kant K[1], Reis RL[2,3,4], Correlo VM[2,3,4], Diéguez L[1]

1. International Iberian Nanotechnology Laboratory (INL) , Braga, Portugal. 2. 3B's Research Group, I3Bs - Research Institute on Biomaterials, Biodegradables and Biomimetics of University of Minho, Headquarters of the European Institute of Excellence on Tissue Engineering and Regenerative Medicine, Guimarães, Portugal. 3. ICVS/3B's–PT Government Associated Laboratory, Braga, Guimarães, Portugal. 4. The Discoveries Centre for Regenerative and Precision Medicine, Headquarters at University of Minho, Guimarães, Portugal.

Abstract:

There is an urgent need for new diagnostic and treatment follow-up tools, which allow the early detection of diseases as well as their continuous monitoring (1). Therefore, during the last years, a new generation of biosensor technologies with improved performance has been developed. The combination of advanced biomaterial methods, biochemical tools and nanotechnology approaches has resulted in the development of innovative 3D biosensors, able to mimic the extracellular cell environment, leading to a more precise diagnosis (1,2). Among the different materials to support 3D biosensors, hydrogel-based systems are considered advantageous, since they provide the ideal conditions within the 3D matrix towards specific sensing (3). Herein, we developed a hybrid novel material, by embedding gold nanostars into gellan gum “spongy-like” hydrogels (GG-SLH). This novel material was developed to be used as surface-enhanced Raman scattering (SERS) substrate for biochemical detection. SERS has demonstrated to be a powerful analytical tool, offering high sensitivity and selectivity, structural information and multiplexing (4). The optical and morphological characterisation of these 3D plasmonic sensors demonstrated the efficient incorporation of NPs into the hydrogel matrix. By taking advantage of the extra SERS enhancement offered by gold nanostars and silver coated gold nanorods, detection limits down to the nanomolar region were achieved using these 3D plasmonic scaffolds. Finally, we demonstrated the potential of these novel 3D hybrid materials as metabolite sensors by showing the detection of lactate, a metabolite that expresses dysregulated levels associated to cancer proliferation.

References:
1. R. Rebelo, et al., Biosens. Bioelectron., 2019, 130, 20–39.
2. R. Edmondson, et al., Assay Drug Dev. Technol., 2014, 12, 207–218.
3. B. Sharma, et al, MRS Bull., 2013, 38, 615.
4. K. Kant, S. Abalde-Cela, Biosensors, 2018, 8, 62.

Promover o desenvolvemento tecnolóxico, a innovación e unha investigación de calidade.
FEDER. Unha maneira de facer Europa. Centro Singular de investigación de Galicia 2016-2019

Poster Communication (PC49)

Analysis of the stability of ternary mixtures of parenteral nutrition with viscosity measures carried out different days after its elaboration

Otero Millán L[1], Lago Rivero N[1], Piñeiro Corrales G[1], Legido Soto JL[2]

1. Hospital Pharmacy Service, Hospital Álvaro Cunqueiro - Instituto de Investigación Sanitaria Galicia Sur. 2. Applied Physics Department, Universitiy of Vigo, Vigo, Spain

Abstract:

Parenteral nutrition (PN) is an essential way of nutrition when oral or enteral nutrition is unable to meet the nutritional requirements. PN are ternary mixtures with a complex composition; they are formed by macronutrients (amino acids, lipids and glucose) and by micronutrients (electrolytes, trace elements and vitamins). Thus, interactions between those components can occur and lead to instability of the mixture compromising its safety.
Preterm infants need this therapy because their gastrointestinal tract is still unable to manage and absorb the nutrients necessary for their development.
In this work we studied the stability of NP mixtures with viscosity measurements.
The samples were prepared by specialized personnel under aseptic techniques in horizontal laminar flow cabinets. A total of 7 different components were calculated to cover the requirements of a premature newborn of 1 kg during the first week of life. The samples are 100 ml and the nutrients are increased in each one.
An Anton Paar AMV 200 viscometer was used to determine the viscosity. To determine the dynamic viscosity, the density was calculated with an Anton Paar DMA4500 densimeter.
The measurements were made on the day +1 and day +7 after the preparation and at 25ºC and 35ºC. The samples were stored in the refrigerator.
No differences were found between the measurements on day 1 and day 7 at 25 ° C ($p = 0.910$) or at 35 ° C ($p = 0.247$).
The samples remain stable during the storage period based on the viscosity measurements.

Promover o desenvolvemento tecnolóxico, a innovación e unha investigación de calidade.
FEDER. Unha maneira de facer Europa. Centro Singular de investigación de Galicia 2016-2019

Poster Communication (PC50)

MCM-41 silica nanoparticles for biological applications

Piñeiro-Redondo Y, Yáñez-Vilar S, Rivas J

Applied Physics Department, NANOMAG Laboratory, Research Tecnological Institute, Universidade de Santiago de Compostela (USC), Spain

Abstract:

Monodisperse SiO2 nanoparticles are one of the most attractive materials because of their unique properties, such as surface structure and functionality (-OH), optical properties, biocompatibility, etc. Surface functionalized SiO2 finds application in electronics, sensors, catalysis, polishing, paints, fillers and pigments. Because of the biocompatible nature and ease of surface modification, amorphous SiO2 nanoparticles are potential tool in medical biotechnology and used in several applications including target drug and gene delivery, molecular bioimaging, labeling of bio-marker of infected cancer and tumor cells, etc [1,2].
Mesoporous MCM-41 is one of the most widely explored materials for drug delivery due to its excellent properties such as high surface area, high thermal stability and narrow pore-size distribution. It has a hexagonal structure with pore diameter range between 2.5 to 6 nm that can be tuned using different cationic surfactants which work as soft templates.
The aim of this work is to present the best conditions to prepare mesoporous MCMC-41 silica nanoparticles suitable for biological applications i.e. nanoparticles with a size between 20 and 300 nm (see figure) and ordered porosity, stable in water suspension allowing further biofunctionalization.
TEM images of MCM-41 nanospheres with different sizes.

Acknowledgements:
This work was supported by the European Commission MADIA project (732678-MADIA-H2020-ICT-2016), and the Consellería de Educación Program for the Development of Strategic Grouping in Materials (AEMAT) at the University of Santiago de Compostela (Grant N. ED431E2018/08), and Program for Consolidation of Reasearch Units of Competitive Reference GRC2017 (Grant N. ED431C 2017/22), Xunta de Galicia.

References:
1. Muhammad F, Gui M, Qi W, Sun F, Wang A, Guo Y, Zhu G, J. Am Chem Soc. 133, 8788 (2011)
2. Bonacchi S, Genovese D, Juris R, Monalti M, Prodi L, Rampazzo E, Zaccheroni N, Angew Chem Int Ed. 50, 4056 (2011)

Promover o desenvolvemento tecnolóxico, a innovación e unha investigación de calidade.
FEDER. Unha maneira de facer Europa. Centro Singular de investigación de Galicia 2016-2019

Poster Communication (PC51)

Bioactive mesoporous nano-size fillers as dielectric biosimilar restorative materials

Yáñez-Vilar S[1], Rivas B[2], Vargas-Osorio Z[1], Piñeiro-Redondo Y[1], Rivas J[1]

1. Applied Physics Department, NANOMAG Laboratory, Research Tecnological Institute, Universidade de Santiago de Compostela (USC), Spain. 2. Surgical and Medical-Surgical Specialties Department, Universidade de Santiago de Compostela (USC), Spain.

Abstract:

Some of the most promising areas in medical research include dental restorations using light cured composite materials, which in the last years have greatly increased for aesthetic procedures due to their easy and fast handling [1].
A new generation of dental restorative materials is emerging under the premise of providing a high degree of similarity with the properties of the natural tissue, in order to allow for a high degree of integration and comparable properties with the natural tissues. Mesoporous silica nano-size fillers, an innovative material used in tissue regeneration applications for its biocompatibility, osteointegration, high stability under strong pH/temperature variations, chemical versatility for surface functionalization or tailorable structural/textural properties, combine many of the requirements for dental tissue restoration.
In this work, hybrid mesoporous silica is proposed as dentine restorative materials, due to the similarity of their textural properties (BET) and dielectric permittivity (ac impedance) to natural tissue. Together with structural, textural and morphological characterization, electrical properties compared to natural dentin samples are presented showing their overall structural similarity.

Acknowledgements:
This work was supported by the European Commission PANA project (Call H2020-NMP-2015- two-stage, Grant N. 686009) and the Consellería de Educación Program for the Development of Strategic Grouping in Materials (AEMAT) at the University of Santiago de Compostela (Grant N. ED431E2018/08), and Program for Consolidation of Reasearch Units of Competitive Reference GRC2017 (Grant N. ED431C 2017/22), Xunta de Galicia.

References:

1. Manhart J, Kunzelmann KH, Chen HY, Hickel R, J. Biomed Mater Res. 53(4), 353-61 (2000)

Promover o desenvolvemento tecnolóxico, a innovación e unha investigación de calidade.
FEDER. Unha maneira de facer Europa. Centro Singular de investigación de Galicia 2016-2019

Poster Communication (PC52)

New index based in anthropometric parameters for the assessments of muscle mass and strength in football players in development

Martinez J[1], Moreira C[1,2], Simón S[1], Quinteiro S[1], Mallo F[1,3]

1. Biomedical Research Centre (CINBIO), University of Vigo, Spain. 2. Universidade do Minho, Braga, Portugal. 3. University of Vigo, Vigo. Spain.

Abstract:

A football player in development remains in football academy since he was 8 years old (benjamin category) until he is 19 years old (youth category). So a normal growth accompanied by physical exercise and constant training will allow him to develop his abilities as player but also to get an optimal body development for performance and health status.
In this work we studied the anthropometry of the players of Celta de Vigo in the under 19 teams, thus football players in development in an elite football academy. Individual characterization also allows to describe the differences between categories and to establish normal ranges of the studied parameters respect age. Anthropometric measurements include: weight, height, skin folds, limb perimeters and muscle grip strength of both hands.
We have calculated percentiles of each relevant parameter respect age or height as needed. For same especially relevant parameters for controlling fitness in football players as the % whole body fat we determine the respective interval of confidence to establish the upper limit. It has been set in 13% for all categories irrespective the age.
In addition we have described new ratios based in the relation of anthropometric measurements, which allows us to evaluate and follow up the increase of muscle mass and to detect and correct imbalances between extremities of both lower and upper limbs. We here proved for the first time that the hand-grip strength is a very good marker of the total muscle strength in players, and that it is related to both the muscle mass of the arm and the leg. In addition, in growing population percentiles for several parameters have been created to determine normal ranges and allow the medical and technical staff on duty to establish individualized strategies to improve the sportive performance of the players in development.

Promover o desenvolvemento tecnolóxico, a innovación e unha investigación de calidade.
FEDER. Unha maneira de facer Europa. Centro Singular de investigación de Galicia 2016-2019

Poster Communication (PC53)

Liraglutide modulates the expression of fibrotic indicators in a model of metabolic liver alteration

Quinteiro S, Toba L, Diz-Chaves Y, Simón S, Martínez J, González Matías L, Mallo F

Biomedical Research Centre, University of Vigo, Vigo, Spain.

Abstract:

Caloric restriction in pregnant mothers hampers the development of the fetus inducing conditions that can lead to the development of metabolic diseases such as liver steatosis, which may progress to steatohepatitis and eventually to liver fibrosis. The glucagon like peptide-1 receptor (GLP1) agonist, liraglutide, may protect and repair the liver in addition to its other numerous functions.
The aim of this work was to assess the effects of maternal perinatal food restriction (MPFR) in markers of profibrosis conditions in rats of different breeding ages, and also whether liraglutide may improve them. Sprague-Dawley pregnant rats were randomly assigned to perinatal food restricted mothers. From GD14 to parturition, pregnant rats were treated with liraglutide or vehicle. At postnatal day 21 and at month 3, male pups were sacrificed and livers were analyzed. We studied the expression of biomarkers of profibrosis conditions by qPCR in the liver. We include expression of fibril proteins (Cola1a1, Fn1 & Acta2), key enzymes for synthetizing precursors of Cola1a1 (P4ha3 & Arg1), and cytokine and inflammation markers (Tgfb1 & Nfkb1). Results were analyzed by two-way ANOVA.
MPFR decreases Acta2 and Nfkb1 expression, and increases P4ha3 in pups at 21 days. Liraglutide reduces expression of Arg1. MPFR with Liraglutide restores control values in Fn1 and Tgfb1. MPFR alters biomarkers expression although not affecting fibrotic pathway in 21 day pups. Liraglutide treatment doesn't affect fibrosis biomarkers expression except for Arg1 in 21 days pups. Alterations induced by MPFR in 21 days pups, were not observed in 3 months rats.

Promover o desenvolvemento tecnolóxico, a innovación e unha investigación de calidade.
FEDER. Unha maneira de facer Europa. Centro Singular de investigación de Galicia 2016-2019

Poster Communication (PC54)

Gelatin magnetic nanobeads for biomedical applications

Teijeiro-Valiño C, González-Gómez MA, Yáñez-Vilar S, Piñeiro Y, Rivas J

NANOMAG Laboratory, University of Santiago de Compostela, Spain.

Abstract:

Gelatin represents a biocompatible, biodegradable and no-immunogenic biomaterial that it is considered as GRAS material (generally recognized as safe) by FDA, in addition to this, gelatin contains rich regions in Arg-Gly-Asp (RGD) that help to boost cell interaction. On the other hand, superparamagnetism of iron oxide magnetic nanoparticles make these particles an attractive tool for different biomedical applications such as magnetic resonance imaging, cell separation and detection, tissue repair or magnetic hyperthermia. The goal of this work was prepare gelatin coated magnetite nanobeads (gelatin magnetic nanobeads) as a versatile platform for biotechnology applications. The gelatin magnetic nanobeads were prepared using an emulsification technique consisting of mixing an oily phase with an aqueous phase under vigorous stirring and sonication. The resulted nanobeads are 230nm in size (measured by TEM) and present a saturation magnetization (Ms) lower than that of bulk magnetite (92 emu/g). Present work is focused on developing smaller gelatin magnetic nanobeads (around 100nm) with different preparation methodologies, using softer conditions that avoid the use of high temperature or sonication during the process which present low compatibility with some therapeutic biomolecules like genetic material or antibodies.

Promover o desenvolvemento tecnolóxico, a innovación e unha investigación de calidade.
FEDER. Unha maneira de facer Europa. Centro Singular de investigación de Galicia 2016-2019

Poster Communication (PC55)

Optimization of a histological technique to detect fatty liver

Rodríguez Alleres G, Rial Otero R, Quinteiro S, Toba L, Diz Chavez Y, Mallo F, Tebar A

Biomedical Research Centre - CINBIO, University of Vigo, Spain

Abstract:

Oil Red O dyeing solution constitutes a staining technique used for the specific detection of lipids in different tissue samples. This permits us to detect the presence of adipose cells and fat after carrying out a histological study.
We here analyzed the liver of three months old pups whose mothers were submitted to perinatal food restriction to verify if diet promoted lipid accumulation in liver cells.
We also analyzed how treatment with liraglutide, an agonist for the GLP-1 receptor agonist, affected to the presence of these cells in the liver. Liraglutide has been described to have different beneficial effects in metabolism in targets as adipose tissue and the liver.
The object of this work was to optimize the dyeing technique for these samples, as the common standardized protocol did provide the expected results initially.
It was developed a protocol for systematically test the staining conditions for Oil Red O (Sigma-Aldrich. 1002223009): preparing mother staining solution and filtering a minimum of three times. After this, let it rest for two days and proceed to prepare the working solution. Working solution was filtering another three times. We tested different timing of sample incubation; 3, 4, 5, 6, 8, 10 and 15 minutes and the better timing was 3.5 minutes. Checking of integrity and final staining of samples under microscope (Nikon Eclipse Ni-E) was doing immediatly an waitting 2, 6 an 12 hours, observing better results when done immediatly. The amplification used was 100x, and analyzed with software NiS Elements.
It was determined that a standardized protocol does not serve us for the tissue studied, since, after looking it under the microscope, we only observed precipitated fat. After the aforementioned modifications, the sample was optimally stained, which allows us to analyze it.

Promover o desenvolvemento tecnolóxico, a innovación e unha investigación de calidade.
FEDER. Unha maneira de facer Europa. Centro Singular de investigación de Galicia 2016-2019

Poster Communication (PC56)

Liraglutide prevents the mitochondrial alteration in hippocampus of pups from food restricted mothers

Simón S, Martínez J, Quinteiro S, Toba L, González-Matías L, Mallo F, Diz Y

Biomedical Research Centre - CINBIO, University of Vigo, Spain.

Abstract:

Maternal perinatal food restriction (MPFR) promotes inadequate development of the placenta, altering nutrient transfer capacity to the foetus. Poor nutrient supply induces intrauterine growth restriction, resulting in low-birth weight, postulated to increase risks of future morbidity. Due to brain's highly energy demand, it constitutes a susceptible organ to malnutrition, being hippocampus highly affected by global malnutrition.
For the study of brain metabolism under MPFR we addressed the study of mitochondrial status, biogenesis and homeostasis in hippocampi of 21-days old male pups.
Pregnant rats were submitted to MPFR and treated with liraglutide (GLP-1 receptor agonist, 100 mg/kg/day sc.), or vehicle (saline). We designed four experimental groups: control mothers fed ad libitum treated with vehicle or liraglutide; and MPFR treated with vehicle or liraglutide.
The mRNA expression of genes involved in mitochondrial biogenesis was quantified by RT-PCR. Peroxisome proliferator-activated receptor gamma co-activator 1-alpha (Ppargc1a), mechanistic target of rapamycin kinase (Mtor), estrogen related receptor alpha (Esrra), sirtuin 1 (Sirt1), transcription factor A mitochondrial (Tfam) and dynamin 1 (Dnm1) were the genes selected.
MPFR caused lower body weight in pups, compared to controls, although liraglutide partially mitigated this diminution. MPFR situations increased the expression levels of Ppargc1a, Mtor, Tfam and Dnm1 and Liraglutide restores levels of Ppargc1a and Mtor, but not of Tfam and Dnm1.
We conclude remarking that maternal perinatal food restriction promotes the expression of factors implied in mitochondria induction, which involve the activation of the Ppargc1a and Mtor but not Sirt1 or Esrra. Liraglutide reverts the activation of Ppargc1a and Mtor with not effect in the markers of mitochondria quantity: Dnm1 and Tfam. Thus, Liraglutide may have neuroprotective effect improving metabolic control of mitochondria in hippocampal cells.

Promover o desenvolvemento tecnolóxico, a innovación e unha investigación de calidade.
FEDER. Unha maneira de facer Europa. Centro Singular de investigación de Galicia 2016-2019

Poster Communication (PC57)

Synthesis and characterization of hybrid inorganic-excitonic core-shell nanoparticles with plasmon-like properties

Estévez-Varela C*, Núñez-Sánchez S, Pastoriza-Santos I

Centro Singular de Investigacións Biomédicas (CINBIO) and Departamento de Química Física, Universidade de Vigo, Vigo, Pontevedra, Spain.* carlestevez@alumnos.uvigo.es

Abstract:

Plasmonic fields can be defined as quantized waves in a collection of mobile electrons which are disturbed from their equilibrium positions by light1. These light-matter interactions are extremely interesting due the high local field obtained at nanoscale. Nanostructured noble metals have been the most widely studied materials in plasmonics due to their optical properties within the UV-vis-NIR spectrum2. However, plasmonic properties have been recently observed in other non-metallic materials such as organic thin films based on J-aggregates3.
The main goal of this work is to obtain stable colloidal dispersions of core-shell nanoparticles composed by a silica core and a J-aggregate shell which can support local surface plasmon-like properties. The role of the silica nanoparticles is being the inorganic scaffold or template for molecular J-aggregates whom will bring the plasmon-like properties to the colloids. In order to achieve that, first silica nanoparticles with size control were obtained following the method proposed by Bogush et al.4. Then, J-aggregates were deposited by Layer-by-Layer technique on the silica surface mainly through electrostatic forces. Three different cyanine dyes have been used as the organic molecule to form J-aggregate shell. The Transmission Electron Microscopy (TEM) images and zeta potential measurements reveals the deposition of the J-aggregates on the surface of the silica nanoparticles. The plasmon-like properties of the hybrid nanoparticles were studied as a function of the number of J-aggregate layers deposited on the silica surface by UV-vis spectroscopy.

References

(1) Xia, Y.; Campbell, D. J. Plasmons: Why Should We Care? *J. Chem. Educ.* **2007**, *84* (1), 91.
(2) Chan, G. H.; Zhao, J.; Schatz, G. C.; Van Duyne, R. P. Localized Surface Plasmon Resonance Spectroscopy of Triangular Aluminum Nanoparticles. *J. Phys. Chem. C* **2008**, *112* (36), 13958–13963.
(3) Gentile, M. J.; Núñez-Sánchez, S.; Barnes, W. L. Optical Field-Enhancement and Subwavelength Field-Confinement Using Excitonic Nanostructures. *Nano Lett.* **2014**, *14* (5), 2339–2344.
(4) Bogush, G. H.; Tracy, M. A.; Zukoski, C. F. Preparation of Monodisperse Silica Particles: Control of Size and Mass Fraction. *J. Non. Cryst. Solids* **1988**, *104* (1), 95–106.

Promover o desenvolvemento tecnolóxico, a innovación e unha investigación de calidade.
FEDER. Unha maneira de facer Europa. Centro Singular de investigación de Galicia 2016-2019

Poster Communication (PC58)

RADAR: R package for RNA-Seq experiment design and analysis

Mendoza M, Canchaya CA

Biomedical Research Centre – CINBIO, University of Vigo, Spain.

Abstract:

RNA-Seq technology arose a decade ago to improve the transcriptome analysis, allowing to study expression levels of transcripts and their isoforms as well as to identify possible coding-sequence mutations. The deluge of information generated by RNA-Seq implies computational challenges (e.g lots of memory and processing time) and many software approaches have been developed to analyse efficiently RNA-Seq data in different computational platforms and programming languages. R is a powerful working environment and programming language for data analysis; nowadays, more than 10,000 packages have been developed for many different purposes (e.g. statistical analysis, big data management). Here, we present the first draft of a new R package for RNA-Seq experiment design and analysis in R (RADAR), which allows us to accomplish the whole RNA-Seq pipeline in R: from experiment design and statistical power estimation to final differential gene expression analysis or gene ontologies enrichment. Using RADAR, we were able to analyse the impact of sample size (number of replicates per group and number of reads) in possible differentially expressed genes in simulated disease-control experiments.

Promover o desenvolvemento tecnolóxico, a innovación e unha investigación de calidade.
FEDER. Unha maneira de facer Europa. Centro Singular de investigación de Galicia 2016-2019

Poster Communication (PC59)

The role of physical activity on healthy brain aging using a wearable device to measure and quantify free-living physical activity

Domingos C, Pêgo JM, Santos NC

Life and Health Sciences Research Institute (ICVS), School of Medicine, University of Minho, Braga, Portugal; ICVS/3B's, PT Government Associate Laboratory, Braga/Guimarães, Portugal

Abstract:

Healthy brain aging is one of the most important determinants of wellness and quality of life in older population. Several studies evidence that physical activity (PA) contributes to brain health. However, the use of subjective measures of PA and the lack of multimodal neuroimaging approaches limited the understanding of this association. This study aims to explore the associations between PA and brain structure and function by objectively evaluating PA with concurrent neuroimaging approaches. For this, a sample of 120 healthy older adults (aged 65 to 75, average 68.4 years (SD = 3.58); 7.93 median years of schooling (SD 5.39); 55.8% female) was recruited from the community, nursing homes, day-care centers, and local gyms. Participants included were 67 females (55.8%), with an average of 68.4 years old (SD = 3.58) and with 7.93 median years of schooling (SD 5.39). A baseline characterization was performed a standardized clinical interview to assess medical history, current medication, general lifestyle-related parameters, functional status and quality of life. Neuropsychological evaluation was administered to assess mood and global cognitive profiles. Participants were scanned on a clinically Siemens 3 T MRI. The imaging protocol include structural acquisitions to assess white and gray matter structures, and functional acquisitions to access brain function at rest. Participants were also asked to wear a Xiaomi Mi Band 2 over a 15-days period to monitor their PA levels. Objective and continuous patterns of PA obtained will be correlated with neuroimaging data. The correlations and further statistical analysis will be performed using SPSS Statistics 24.0 and data mining algorithms. Using this innovative approach, we expect to provide a better understanding on the mechanisms underlying how PA may protect brain aging on healthy older adults.

Promover o desenvolvemento tecnolóxico, a innovación e unha investigación de calidade.
FEDER. Unha maneira de facer Europa. Centro Singular de investigación de Galicia 2016-2019

Poster Communication (PC60)

RKIP protein as a novel predictor of EGFR targeted therapies in lung cancer

Raquel-Cunha A, Pinheiro J, Cardoso-Carneiro D, Freitas G, Reis RM, Martinho O

1. Life and Health Sciences Research Institute (ICVS), School of Medicine, University of Minho, Campus Gualtar, Braga, Portugal. 2. ICVS/3B's - PT Government Associate Laboratory, Braga/Guimarães, Portugal. 3. Molecular Oncology Research Center, Barretos Cancer Hospital, Pio XII Foundation, Barretos, S.Paulo, Brazil

Abstract:

Lung cancer (LC) is one of the most fatal cancers in the world with life expectancy rarely reaching five years. Molecular targeted therapies have been greatly developed with EGF receptor inhibitors outstanding. Unfortunately, patients end up developing drug resistance, hence, it is essential to discover novel predictors of therapy response. In this sense, Raf kinase inhibitory protein (RKIP) arises, as it is an important regulator of relevant intracellular signalling pathways (MAPK). Considered to be a metastasis suppressor, its downregulation was associated with tumour malignancy and poor prognosis, in several tumour types, including LC. Herein, the aim was to understand the role of RKIP in the modulation of tumour cells response to anti-EGFR therapies.

To do so, RKIP knockout (KO) was made in three LC cell lines, H292, A459 and PC9. The influence of RKIP's loss of expression was evaluated by cytotoxicity assays, to compare the efficiency of different tyrosine kinase (TK) inhibitors (Erlotinib, Afatinib, AST1306 and Osimertinib). Western blot analysis was done to identify which pathways were being affected by the inhibition of these drugs.

Throughout the distinct LC cell lines, we observed that, upon RKIP KO, EGFR overactivation is induced. Also, as expected, the same KO clones tend to become more resistant to TK inhibitors when compared to their controls. Further analysis revealed that upon loss of RKIP expression, proteins intervening in important carcinogen pathways, such as MAPK, are overexpressed. This might be the path to the observed gain of resistance.

Thus, our results suggest that RKIP can modulate MAPK pathway and render tumour cells less sensitive to drugs. With all in consideration, low levels of RKIP correlate with a negative response to EGFR targeted therapies, meaning that RKIP may be a potential biomarker of negative response in lung cancer treatment.

Promover o desenvolvemento tecnolóxico, a innovación e unha investigación de calidade.
FEDER. Unha maneira de facer Europa. Centro Singular de investigación de Galicia 2016-2019

FEDER. Unha maneira de facer Europa. Promover o desenvolvemento tecnolóxico, a innovación e unha investigación de calidade. Centro Singular de Investigación de Galicia 2016-2019.