ABSTRACT BOOK

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Oral Presentations

Wednesday, November 16

8:30- 9:00 AM **MISEV2022 and EV-TRACK**

9:00- 9:15 AM **ISEV Rigor Task Forces**

9:15- 9:40 AM

Single-molecule assay for the biophysical characterization of extracellular vesicles

Andras Saftics[1], Sarah Abu-Elreich[1], Eugenia Romano[1], Ima Ghaeli[1], Kathleen M. Lennon[1], Gagandeep Singh[2], Debbie C. Thurmond[3], Saumya Das[4], Kendall Van Keuren-Jensen[5], Tijana Jovanovic-Talisman[1]

[1]Department of Cancer Biology and Molecular Medicine, Beckman Research Institute, City of Hope Comprehensive Cancer Center, Duarte, CA, USA ~~2~~Department of Surgery, City of Hope Comprehensive Cancer Center, Duarte, CA, USA [3]Department of Molecular and Cellular Endocrinology, Diabetes and Metabolism Research Institute, Beckman Research Institute of City of Hope, Duarte, CA, USA [4]Cardiology Division and Corrigan Minehan Heart Center, Massachusetts General Hospital, Harvard Medical School, Boston, MA, USA. [5]Neurogenomics Division, Translational Genomics Research Institute, Phoenix, AZ, USA

Extracellular vesicles (EVs) are an excellent source of biomarkers. However, due to the challenges in robust isolation and characterization of specific EV populations, the clinical implementation of EV-based diagnostics is still not fully established. To address some of these challenges, we combined affinity isolation with quantitative single-molecule localization microscopy (qSMLM), a powerful imaging method that can simultaneously assess EV size, molecular content, and heterogeneity. Here, we present a novel qSMLM-EV assay for the characterization of EV subpopulations from human plasma samples. We first applied the assay to size exclusion chromatography isolated EVs. The number of detected tetraspanin-enriched EVs positively correlated with sample dilution in a 64-fold dilution range. We further characterized the size and molecular tetraspanin content of CD9-, CD63-, and CD81-enriched EV populations. Moreover, we have shown that the qSMLM-EV assay can be directly applied to crude plasma samples; the number of detected EVs positively correlated with sample dilution in a 50-fold dilution range. Finally, we assessed how size exclusion chromatography isolation affected EV size, molecular content of tetraspanins, heterogeneity, and EV yield. Our ultimate goal is to further develop this qSMLM-EV assay into a platform for characterizing disease-associated and organ-associated EVs.

9:40- 10:00 AM

Light Scattering-Based Analytics of EVs: a powerful set of techniques -- but with great power comes great caution

Wyatt N. Vreeland, National Institute of Standard & Technology

Many analytical techniques used to measure EV size and count are fundamentally based on light scattering. The light scattering is used to detect EVs (and generate an EV number count) and various qualities of the scattered light are used to determine EV size. Techniques that are light scattering-based include: Dynamic Light Scattering (DLS) (also known as Quasi-Elastic Light Scattering (QELS) and Photon Correlation Spectroscopy (PCS)), Nanoparticle Tracking Analysis (NTA) (also known as Particle Tracking

Analysis (PTA)), and Multiangle Light Scattering (MALS). The primary literature describing the relation between light scattering patterns and particles characteristics is extremely dense and only accessible to experts after years of study.

In this presentation, I will simplify what light scattering is really reporting, what assumptions the data analysis generally apply and how the reported values of EV size and count can be skewed and possibly lead to incorrect conclusions. The concepts will be presented in plain language and accessible to the whole EV research community -- I promise no physics jargon or crazy equations.

10:30- 11:00 AM

Bulk to Single vesicles characterization

Juan M Falcon-Perez, Center for Cooperative Research in Biosciences (CIC bioGUNE), Basque Research and Technology Alliance (BRTA), CIBERehd

Extracellular vesicles (EVs) are heterogeneous lipid containers with a complex molecular cargo comprising several populations with unique roles in biological processes. These vesicles are closely associated with specific physiological features, which makes them invaluable in the detection and monitoring of various diseases. EVs play a key role in pathophysiological processes by actively triggering genetic or metabolic responses. However, the heterogeneity of their structure and composition hinders their application in medical diagnosis and therapies. This diversity makes it difficult to establish their exact physiological roles, and the functions and composition of different EV (sub)populations. Ensemble averaging approaches currently employed for EV characterization, such as western blotting or 'omics' technologies, tend to obscure rather than reveal these heterogeneities. Recent developments in single-vesicle analysis have made it possible to overcome these limitations and have facilitated the development of practical clinical applications. In this review, we discuss the benefits and challenges inherent to the current methods for the analysis of single vesicles and review the contribution of these approaches to the understanding of EV biology. We describe the contributions of these recent technological advances to the characterization and phenotyping of EVs, examination of the role of EVs in cell-to-cell communication pathways and the identification and validation of EVs as disease biomarkers. Finally, we discuss the potential of innovative single-vesicle imaging and analysis methodologies using microfluidic devices, which promise to deliver rapid and effective basic and practical applications for minimally invasive prognosis systems.

10:50- 11:10 AM

Asymmetric depth filtration: A versatile and scalable method for high-yield isolation of extracellular vesicles with low contamination

Mikhail Skliar, University of Utah

We developed a novel asymmetric depth filtration (DF) approach to isolate extracellular vesicles (EVs) from biological fluids that outperforms ultracentrifugation and size-exclusion chromatography in purity and yield of isolated EVs. By these metrics, a single-step DF matches or exceeds the performance of multistep protocols with dedicated purification procedures in the isolation of plasma EVs. We demonstrate the selective transit and capture of biological nanoparticles in asymmetric pores by size and elasticity, low surface binding to the filtration medium, and the ability to cleanse EVs held by the

filter before their recovery with the reversed flow all contribute to the achieved purity and yield of preparations. We further demonstrate the method's versatility by applying it to isolate EVs from different biofluids (plasma, urine, and cell culture growth medium). The DF workflow is simple, fast, and inexpensive. Only standard laboratory equipment is required for its implementation, making DF suitable for low-resource and point-of-use locations. The method may be used for EV isolation from small biological samples in diagnostic and treatment guidance applications. It can also be scaled up to harvest therapeutic EVs from large volumes of cell culture medium.
https://onlinelibrary.wiley.com/doi/full/10.1002/jev2.12256

11:10- 11:30 AM

From Labeling, Dosage, Size to Biodistribution and Function of Extracellular Vesicles

Charles Pin-Kuang Lai[1,2,3]

[1]Institute of Atomic and Molecular Sciences, Academia Sinica, Taipei, 10617 Taiwan; [2]Chemical Biology and Molecular Biophysics Program, TIGP, Academia Sinica, Taipei, 11529 Taiwan; [3]Genome and Systems Biology Degree Program, National Taiwan University, Taipei, 10617 Taiwan

Extracellular vesicles (EVs) are varying sized bionanoparticles released by cells to mediate intercellular communication under (patho)physiological conditions. While a majority of EV-related studies focuses on small EVs (sEVs; e.g., exosomes; <100 - 200 nm), less research examines medium and large EVs (m/lEVs; e.g., microvesicles; >200 nm) and their functions. Meanwhile, a high dose of EV is commonly applied to exert a phenotype in vivo, often as an attempt to detect dye-labeled EVs which yields a low signal-to-noise signal. We thus investigated key parameters of EV in vivo study such as labeling methods, EV sizes, as well as their subsequent biodistribution and biological function. We compared biophysical and in vivo property of EVs labeled with lipophilic dyes and our latest bioluminescence resonance energy-based EV reporter, PalmGRET. DiR significantly alters the size and biodistribution of the labeled EVs when compared to PalmGRET-labeled counterparts. Notably, a high dose of DiR-EV (50 – 100 μg per mouse) is required to attain an appreciable signal when administered intravenously, and is often lethal. By contrast, PalmGRET-EVs can be readily detected for sensitive biodistribution analysis at a non-lethal and physiological dose (5 – 10 μg). Importantly, sEV and m/lEVs comprise of varying sized EV populations. Using PalmGRET-EVs at the physiological dosage, we observed a differential biodistribution between sEV and m/lEV derived from 4T1 breast cancer, and further demonstrated their protumorigenic potential in breast cancer-bearing mice. To the best of our knowledge, this is the first study which empirically optimized the labeling method and dosage for EV in vivo study, and subsequently revealed distinct biodistribution profile and protumorigenic function of both sEV and m/lEVs.

Thursday, November 17

8:00- 9:00 AM

A versatile toolbox for a comprehensive view on extracellular vesicles

An Hendrix, Ghent University

Knowledge on the origin, fate and function of extracellular vesicles (EVs) in the human body is required to accelerate therapeutic and diagnostic applications but hampered by a plethora of technological pitfalls (PMID: 33568799; PMID: 30975688). We created the EV-TRACK knowledgebase

(http://evtrack.org) to stimulate transparency and steer reproducibility (PMID: 28245209). We established usable and reproducible protocols to separate EVs from other extracellular particles in body fluids, including blood plasma, stool and urine
(PMID: 34429857; PMID: 31776460; PMID: 32284825; PMID: 33111109). To support instrument calibration and data normalization we designed recombinant EVs that are easily trackable and distinguishable from sample EVs (PMID: 31337761; PMID: 33452501). This in-house developed know-how steered my research group towards the discovery of systemically circulating bacterial EVs (BEVs) in cancer patients (PMID: 30518529; PMID: 35033427).

9:15- 9:30 AM

Production of immunomodulatory extracellular vesicles (EVs) from human bone marrow-derived mesenchymal stromal cells (hMSCs) using a hollow-fibre bioreactor system under varying flow rates and seeding densities.

Imen Gdara, Abidemi Olayinka, Dimitrios Tsiapalis, Tobias Tertel, Verena Boerger, Lorraine O'Driscoll, Bernd Giebel, Paul A. Cahill

Extracellular vesicles (EVs) produced by human bone marrow-derived mesenchymal stromal cells (hMSCs) hold great promise for their therapeutic potential in several immune-mediated diseases, including COVID-19. The large amount of hMSC-derived EVs required for clinical testing necessitate the deployment of a scalable bioreactor production system for manufacturing these biologics for therapeutic use in humans. To address the potential utility of the Fibercell™ hollow-fibre bioreactor system (HFBR) for scalable production of EVs, hMSCs from 3 x donors were inoculated into C2025D and C2011 bioreactors that accommodate a surface area of 450 cm2 and 4,000 cm2 and a volume of 2.8mls and 20mls, respectively within the extra-capillary space (ECS). The hMSCs were maintained within the ECS in a chemically defined GMP compliant serum free media (CDM-HD) while the circulating medium that passed through the intracapillary space (ICS) at various flow rates (5 - 40mls/min) was supplemented with 10% human platelet factor (hPL). The EVs released into the ECS were characterised over time for size, concentration, immunophenotype and immunomodulatory activity. The concentration of EVs released within the ECS was confirmed using HEK-293T-CD-63-GFP cells where the molecular weight cut-off of each fibre within the HFBR facilitated the transport of nutrients and waste products through the hollow fibres but concentrated the EVs within the ECS. HFBRs inoculated with hMSCs and maintained at a flow rate of 15mls/min exhibited high viability over 150 days in culture and retained their expression of mesenchymal associated markers (CD44, CD73, CD90, CD105) upon cell retrieval after 10 weeks. The hMSC-EVs released within the ECS displayed consistency in size and concentration in addition to presenting a reproducible EV surface immunophenotype for CD9, CD81 and CD63. Increasing flow rates resulted in a significant decrease in the number of CD81 and CD63 EVs released into the ECS in both low and high density cultures. The immunomodulatory functionality of the released EVs under low and high flow conditions was confirmed using a mixed lymphocyte reaction (MLR) assay. We conclude the Fibercell™ HFBR production systems are suitable for the reproducible scalable GMP production of functionally active hMSC-derived EVs.

9:30- 9:45 AM

Scalable production of human induced Pluripotent Stem Cell-derived Extracellular Vesicles in Stirred-tank Bioreactors

Ana Filipa Louro[1,2] Ana Meliciano[1,2] Pedro Vicente[1,2] Cláudia Diniz[1,2] Paula M. Alves[1,2] Margarida Serra[1,2]

[1]iBET, Instituto de Biologia Experimental e Tecnológica, Oeiras, Portugal; [2]Instituto de Tecnologia Química e Biológica António Xavier, Universidade Nova de Lisboa, Oeiras, Portugal

A significant bottleneck in the advancement of Extracellular Vesicle (EV)-based therapies is the efficient large-scale manufacture of clinical-grade EV. Upstream challenges include the selection of an appropriate EV parent cell and its large-scale expansion. We previously identified human induced Pluripotent Stem Cells (hiPSC) as a source of native EV for cardiac regeneration and pinpointed the bioactive signatures of hiPSC-EV. Currently, we are developing scalable bioprocesses for hiPSC expansion in Stirred-tank Bioreactors (STB) and modulating critical process parameters to intensify EV production. Briefly, hiPSC were expanded as 3D aggregates in STB (DasGip Eppendorf AG), operated in perfusion (D=1.3 day-1). Throughout hiPSC expansion, the dissolved O2 concentration was controlled at low levels (4% O2). EV were isolated by density gradient ultracentrifugation, probed for specific markers, and characterized in terms of particle size distribution and morphology. EV bioactivity was assessed in human umbilical vein endothelial cells. A 2.9-fold increase in cell concentration was observed in STB compared to the static 2D monolayer culture, which resulted in a 3.1 increase in total particles isolated per mL of conditioned medium. hiPSC-EV produced in STB presented a cup-shape morphology and were positive for EV markers. Tube formation assays showed increased pro-angiogenic activity for hiPSC-EV produced in STB versus static 2D monolayer culture. Overall, our study validates hiPSC as cell biofactories for large-scale EV production in STB and provides insights into manufacturing EV-based products. Ongoing work aims at optimizing EV production yields and potency by further manipulating process parameters such as dissolved O2, stirring rate and operation mode. Funding: FCT PhD fellowships SFRH/BD/145767/2019 and UI/BD/151255/2021; CARDIOPATCH Interreg SUDOE (SOE4/P1/E1063), BRAV3 (H2020-SC1-BHC-874827); iNOVA4Health (UIDB/04462/2020; UIDP/04462/2020); LS4FUTURE (LA/P/0087/2020).

9:45- 10:00 AM

Comparative analysis of tangential flow filtration and ultracentrifugation, both combined with subsequent size exclusion chromatography, for the isolation of small extracellular vesicles

Keko'olani Visan[1] Andreas Möller[1]

[1]QIMR Berghofer Medical Research Institute, Tumour Microenvironment Laboratory, Herston, QLD 4006, Australia

Small extracellular vesicles (sEVs) provide major promise for advances in cancer diagnostics, prognostics and therapeutics, ascribed to their distinctive cargo reflective of pathophysiological status, active involvement in intercellular communication, as well as their ubiquity and stability in bodily fluids. As a result, the field of sEV research has expanded exponentially. Nevertheless, there is a lack of standardisation in methods for sEV isolation from cells grown in serum-containing media. The majority of researchers use serum-containing media for sEV harvest and employ ultracentrifugation as the primary isolation method. Ultracentrifugation is inefficient as it is devoid of the capacity to isolate high sEV yields without contamination of non-sEV materials or disruption of sEV integrity. We comprehensively evaluated a protocol using tangential flow filtration and size exclusion chromatography

to isolate sEVs from a variety of human and murine cancer cell lines, including HeLa, MDA-MB-231, EO771 and B16F10. We directly compared the performance of traditional ultracentrifugation and tangential flow filtration methods, that had undergone further purification by size exclusion chromatography, in their capacity to separate sEVs, and rigorously characterised sEV properties using multiple quantification devices, protein analyses and both image and nano-flow cytometry. Ultracentrifugation and tangential flow filtration both enrich consistent sEV populations, with similar size distributions of particles ranging up to 200 nm. However, tangential flow filtration exceeds ultracentrifugation in isolating significantly higher yields of sEVs, making it more suitable for large-scale research applications. Our results demonstrate that tangential flow filtration is a reliable and robust sEV isolation approach that surpasses ultracentrifugation in yield, reproducibility, time, costs and scalability. These advantages allow for implementation in comprehensive research applications and downstream investigations.

10:30- 10:45 AM

Development of size-selective capture and release nanopocket membranes for the isolation of extracellular vesicles

Thomas Gaborski, RIT Munther Alsudais, RIT Aslan Dehghani, Sartorius Stedim

The use of nanoscale extracellular vesicles (EV) for diagnostic and therapeutic applications has seen a major interest increase in recent years because of their capacity to exchange components such as nucleic acids, lipids, and proteins between cells. Currently, the “gold standard” method for isolating EVs from biofluids is ultracentrifugation, which requires large volumes of biofluid, long processing times, expensive instrumentation, and trained technicians. Gel precipitation and size exclusion chromatography approaches have been developed that remove the need for ultracentrifugation, but these methods suffer from low yield and/or contamination with co-precipitated proteins. We have recently demonstrated a new method termed tangential flow for analyte capture (TFAC) using ultrathin silicon-based nanomembranes to purify extracellular vesicles from complex biological fluids such as blood plasma and cell culture media. In this method, EVs and similarly sized analytes are captured on the pores of an ultrathin membrane where they can be washed and released. Fundamentally, TFAC resembles bind and elute purification strategies although it distinguishes itself from affinity and ion-exchange chromatography because the binding is purely physical. In order to increase the specificity of EV capture including the separation of different EV sub-populations based on size and surface markers, we are developing a series of nanopocket membranes. We use nanosphere lithography and traditional MEMS approaches to fabricate nanoscale (50-500 nm) pockets, each with a pore, on the surface of a membrane. Nanopockets can be fabricated with varying radii and depth as well as surface properties akin to size and affinity chromatography. These properties can be tailored to sizes of different EV subpopulations, where a series of different nanopocket sizes could capture, fractionate and purify EV populations from complex biofluids such as plasma.

10:45- 11:00 AM

Characterizing ATP1A3 as a novel target for isolating neuron-specific extracellular vesicles from human brain and cerebrospinal fluid

Yang You[1], Zhengrong Zhang[1], Nadia Sultana[2,3], Maria Ericsson[4], Min Sun[5], Yuka A. Martens[1,6], Seiko Ikezu[1], Scott A. Shaffer[2,3], Guojun Bu[1,6], Tsuneya Ikezu[1]

[1]Department of Neuroscience, Mayo Clinic, Jacksonville, FL, USA; [2]Department of Biochemistry and Molecular Biotechnology, University of Massachusetts Chan Medical School, Worcester, MA, USA; [3]Mass Spectrometry Facility, University of Massachusetts Chan Medical School, Shrewsbury, MA USA; [4]Department of Cell Biology, Harvard Medical School, Boston, MA, USA; [5]Nanoview Biosciences, Boston, MA, USA; [6]Center for Regenerative Medicine, Neuroregeneration Laboratory, Mayo Clinic, Jacksonville, FL, USA

Most studies attempted to enrich putative neuron-derived EVs (NDEVs) by immunocapturing the presumed neural marker L1CAM or NCAM1 and characterize their contents as a pathological reflection of the central nervous system. However, the reliability of using them as a marker of brain neuronal EVs has been questioned. We have recently identified the Na+, K+-ATPase (ATP1A3), a transmembrane ion pump, as a potential NDEV marker in an unbiased proteomics study. Here we isolated EVs derived from human induced pluripotent stem cell-derived excitatory neurons (iNeurons), human brain and cerebrospinal fluid (CSF). Immuno-transmission electron microscopy and single particle-interferometric reflectance imaging sensor (SP-IRIS) analysis showed ATP1A3 is more frequently co-localized with tetraspanins on the EVs surface as compared to NCAM1 or L1CAM at a single particle level. In addition, we observed the higher enrichment of ATP1A3 in the EVs from both brain and CSF samples than NCAM1 and L1CAM by immunoblotting, which were significantly diminished after treatment of EVs with proteinase K. Furthermore, we used affinity capture method to separate ATP1A3-associated EVs from total brain or CSF EVs. Label-free mass spectrometry determined a higher enrichment of neuron-specific proteins, including SNAP25, SYT1 and CAMK2A in ATP1A3+ brain-EVs over NCAM1+ or L1CAM+ brain-EVs. Gene set enrichment analysis demonstrated that proteins in ATP1A3+ brain-EVs were significantly associated with myelin sheath, synapse, neurodegenerative diseases, and neuronal cell type, suggesting ATP1A3-associated EVs representatively reflect the neuron origin in the brain. Finally, we showed the presence of AD marker proteins including amyloid-β peptide 1-40 (Aβ40) and 1-42 (Aβ42), total tau, p-tau at T181 (P-tau181) and the neuronal protein SNAP25 in ATP1A3+ CSF-EVs using high-sensitivity digital ELISA (Simoa), demonstrating ATP1A3-associated EVs from CSF as the promising source for AD biomarkers. In summary, our results propose ATP1A3 as a novel target to enrich NDEVs from human samples and provide a new avenue to study molecular changes associated with neurological diseases in the CNS.

11:00- 11:15 AM

EV-AAVs as a Novel Gene Delivery Vector to the Heart: New Assessments for EV-AAVs and free AAV Separation

Sabrina La Salvia[1], Xisheng Li[1], Yaxuan Liang$_2$, Marta Adamiak[1], Erik Kohlbrenner[3], Estrella Lopez-Gordo[1], Seonghun Yoon[1], Prabhu Mathiyalagan[1], Shweta Lodha[1], Anh Phan[1], Nikhil Raisinghani[1], Shihong Zhang[1], Edgar Gonzalez Kozlova[6], , Nicole Dubois[7 8], Navneet Dogra[6], Roger J. Hajjar[9], Susmita Sahoo[1]*

Adeno-associated viruses (AAVs) are one of the best vectors to deliver genes to the heart due to high transgene expression and safety in clinics. However, pre-existing AAV neutralizing antibodies (NAbs) in patients binds to AAVs, prevent efficient gene transduction and limits the number of patients eligible for gene therapy. Here, we have shown that extracellular vesicle-encapsulated AAVs (EV-AAVs) are a superior cardiac gene delivery vector that delivers more genes and offers higher NAb resistance comparted to free-AAVs. Presence of free AAV contaminants in EV-AAV preparations may reduce effective EV-AAV dosing, lower their NAb resistance, and cause unwanted side effects. Therefore, novel

methods are required to efficiently remove free AAVs from EV-AAV preparations to improve their efficacy. Methods and Results: We designed a novel, two-step iodixanol density-gradient ultracentrifugation method (UC+DG) to purify EV-AAVs, free of AAV contamination. We comprehensively characterized the isolated EV-AAVs for their morphology, presence of EV specific markers, size, concentration and AAV genome titer using WB, nano (EV)-flow cytometry, tunable resistive pulse sensing, qPCR, TEM, DLS, ExoView chip analysis and RNAseq. To develop a scalable EV-AAV production for clinical applications, we compared the UC+DG method with size exclusion chromatography (SEC) for vector enrichment, reproducibility, scalability and ease of use. Our results demonstrated NAb evasion and therapeutic efficacy of EV-AAVs. Intramyocardially injected EV-AAV9-SERCA2a, a known therapeutic cardiac gene, to post- infarcted (LAD ligated), pre-immunized mouse hearts significantly improved ejection fraction and fractional shortening compared to free AAV9-SERCA2a delivery. Using PKH67-fluorescent dye or pH sensitive (CypHer) dye, we show that EV-AAVs were internalized into acidic endosomal compartments before reaching the nucleus in human cardiomyocytes. Conclusion: We demonstrated efficient separation of EV-AAVs from free AAVs using UC+DG, as a proof-of-concept, and SEC as a scalable method. We also show significantly higher potency and therapeutic efficacy of EV-AAVs compared to free AAVs. Our results establish the potential of EV-AAVs as a state-of-the art gene delivery vector to treat heart failure.

11:15- 11:30 AM

Developing engineered Extracellular vesicles (eEVs) as a targeted delivery screening platform

Yuki Kawai-Harada[1,2], Hiroaki Komuro[1,2], Katherine Lauro[1,2], Daniel Woldring[1,3], Masako Harada[1,2]

1Institute for Quantitative Health Science and Engineering (IQ), Michigan State University, Michigan, USA 2Department of Biomedical Engineering, Michigan State University, Michigan, USA 3Department of Chemical Engineering and Materials Science, Michigan State University, Michigan, USA

Introduction: Nanocarrier-mediated targeted delivery applications have tremendous potential in providing efficient therapeutics, which is often a critical challenge for treating numerous diseases. Despite the great effort in developing efficient cargo and targeted ligands, both systems suffer in clinical translation, mainly due to insufficient efficacy, toxicity, and strategic reasons. Here, Extracellular vesicles (EVs) hold tremendous potential for drug delivery applications due to their natural capacity in intercellular communication to transport molecules in the body. We are exploring engineered EVs (eEVs) as a novel screening platform for the tissue-specific monobody-EVs using EV-surface display technology. Monobody is a clinically approved fibronectin type III domain antibody mimetics initially developed in the yeast screening system. Methods: We investigated if eEV can serve as a molecular screening platform using transient transfection of monobody library plasmids into HEK293T cells. The characterization of eEVs includes size distribution, DNA/Protein contents, and the capacity of enriched delivery using control monobody and targeted monobody using eEVs generated with mixed DNA ratio in vitro. Results: We have established a method to generate eEVs to package monobody library-coding plasmids and display monobody on their surface, retrieve and quantify monobody-coding sequences from the cells, and re-clone the retrieved monobody to construct the next generation of libraries for repeated screening. Further, we have tested and validated the system to store eEVs to keep their integrity and protect DNA from degradation. Summary: This study revealed the potential of eEVs as a platform for molecular screening, which will advance to establish in vivo screening of eEV-based monobody display libraries.

Friday, November 18

8:00- 9:00 AM

Digital flow cytometry for single-EV analysis

Daniel T. Chiu, University of Washington

We have developed a multi-parametric high-throughput flow-based method for the analysis of individual extracelluar vesicles and particles (EVPs), which are highly heterogeneous and comprise a diverse set of surface protein markers as well as RNA cargoes. Yet, current approaches to the study of EVPs lack the necessary sensitivity and precision to fully characterize and understand the make-up and the distribution of various EVP subpopulations that may be present. We termed our platform digital flow cytometry, which provides single-fluorophore sensitivity to enable phenotyping single EVPs with unprecedented precision and sensitivity.

9:15- 9:30 AM

Correlative light and electron microscopy in EV research

Kirsi Rilla, University of Eastern Finland, Finland

One of the most challenging objectives of extracellular vesicle (EV) research is to learn to combine complementary characterization techniques. Despite multiple advanced imaging methods available to detect and characterize EVs, each imaging technique has its limitations. Light microscopy has restrictions because the smallest EVs are below the resolution of light microscopy. Electron microscopy (EM) cannot image EVs in their native state because the samples need to be fixed and processed before imaging. Thus, there is an increasing demand to study the same samples with different techniques. One of the most promising methods to solve these challenges is correlative light and electron microscopy (CLEM), which combines the labeling power of fluorescence imaging and the high-resolution structural information provided by electron microscopy. We have optimized a straightforward CLEM protocol to combine fluorescent and ultrastructural information by using confocal microscopy and scanning EM. We utilized gridded coverslips for relocation of the cells or EVs quickly and reliably. Our studies demonstrated that fluorescently labeled single EVs even less than 100 nm in diameter can be reliably detected with high-resolution confocal imaging. The correlation of a fluorescent signal with corresponding EM images confirms that the fluorescent signal originates from a vesicle, and is not an artifact of an unspecific signal, which is a high risk in EV-related fluorescent techniques. The disadvantage of the CLEM method is the occasional shrinking of the cellular structures during sample processing for EM which may impede the accurate overlaying of the images. Another drawback of the method is that it is a time-consuming technique and not suitable for high content analysis. This is a simple and cost-effective CLEM method suitable for single EV characterization, release mechanisms of EVs, and their interactions with target cells. The method can be reliably utilized for detailed localization of proteins and morphology of EVs at a single vesicle level and provides more convincing results than either of the techniques alone.

9:30- 9:45 AM

Combined fluorescence and atomic force microscopy for multiparametric profiling of single extracellular vesicles.

Sara Cavallaro[1,2], Federico Pevere[3], Fredrik Stridfeldt[4], André Görgens[5], Carolina Paba[3], Siddharth S. Sahu[3], Doste R. Mamand[5], Dhanu Gupta[5], Samir El Andaloussi[5], Jan Linnros4, Apurba Dev[3,4].

[1]Cancer Center Research, Massachusetts General Hospital, Boston, MA 02129, USA [2]Department of Medicine, Harvard Medical School, Boston, MA 02115, USA [3]Department of Electrical Engineering, Uppsala University, Uppsala 75121, Sweden [4]Department of Applied Physics, KTH Royal Institute of Technology, Stockholm 10691, Sweden [5]Clinical Research Center, Department of Laboratory Medicine, Karolinska Institutet, Stockholm 17177, Sweden.

Increasing efforts have been directed towards the understanding of the biogenesis and functions of extracellular vesicles (EVs). These aspects might open new opportunities for the use of EVs in disease diagnostics and drug delivery. The bulk techniques used for EV analysis (e.g. western blot, electrochemical/colorimetric detection, etc.) can only measure the average properties of the vesicles. Therefore, to fully resolve EV heterogeneity and identify possible EV subpopulations, a single particle technique is needed. However, this type of analysis is complicated by the small size of many vesicles (<100 nm) and their soft nature. These challenges require the use/development of sensitive techniques for single EV analyses. In this work, we show the development of a platform for accurate multiparametric analysis of single EVs. The method combines fluorescence (FL) and atomic force microscopy (AFM) to simultaneously measure and correlate the size, protein expression and mechanical properties of single vesicles. In particular, FL microscopy is used to measure the protein content of the vesicles. High-resolution AFM is instead used to measure the EV diameter over their entire size range, overcoming the limitations of the diffraction limited systems. Moreover, the technique offers the unique ability to provide information about the EV mechanical properties (stiffness), not correlated before on single vesicles. We will show the application of the presented combined method on small EVs (sEVs) isolated from different cell lines. A panel of tetraspanins, protein usually present on sEVs, will be investigated and the correlation between size, protein abundance and stiffness on single vesicles will be presented. The results will show the presence of both common and cell line-specific sEV subpopulations bearing distinct distributions of proteins and biophysical properties.

9:45- 10:00 AM

On-Chip Multimodal NanobioAnalytical Platform for biophysical analysis of Extracellular Vesicle

Geetika Raizada[1], Benjamin Brunel[1], Eric Lesniewska[2], Celine Elie-Caille[1], Wilfrid Boireau[1]

[1]FEMTO-ST Institute, CNRS UMR-6174, Université de Bourgogne Franche-Comté, 15B Av. des Montboucons, 25030 Besançon, France; [2]Interdisciplinary Lab Carnot Bourgogne LICB, CNRS UMR-6303, Université de Bourgogne Franche-Comté 9 Av. Alain Savary, 21078 Dijon, France

Extracellular vesicles (EVs) domain represents a promising field of interest due to their functional biological activities, especially intercellular communication and their release in biofluids, making them promising biomakers for diagnosis or therapeutic follow-up. Many (pre)-analytical challenges must be faced in order to overcome the difficulty of measuring their inherently complex properties, such as polydispersity in size and cellular origin, dynamics of their release and uptake in very complex media... In our group, we have recently developed an in-house NanoBioAnalytical (NBA) platform which can assist in the characterization of EVs subpopulations1, 2. The platform brings detection of EVs in a multiplex format, based on Surface Plasmon Resonance Imaging (SPRi) and Atomic Force Microscopy (AFM), which gives us the metrology, size distribution and morpho-mechanical properties of the

different subsets. Originality of the hyphenated approach is brought by a common substrate, a gold chip, that allow a very in situ, accurate and sensitive EVs characterization. During the conference, the power of this analytical platform will be illustrated through the exploration of particular EVs samples coming from patients in the field of cancer. The tunable property of NBA platform offers further possibilities of biophysical characterizations, particularly the opportunity to couple with Raman spectroscopy by using an original gold biochip as a new core of this platform3. Preliminary Raman tests on such biomaterials give encouraging results which should open up a fruitful discussion during the conference on the ability to measure sequentially the characteristics and the biomolecular profiling of unaltered EVs on the same substrate. 1. S. Obeid et al., NanoBioAnalytical characterization of extracellular vesicles in 75-nm nanofiltered human plasma for transfusion: a tool to improve transfusion safety. Nanomedicine: Nanotechnology, Biology, and Medicine, Vol 20, 101977, 2019 2.B. Namasivayam. Thesis: Establishing the performances of the NanoBioAnalytical platform for extracellular vesicles detection and characterization: Exploration of neuroprotective human platelet lysates (2021). 3. G. Raizada et al., Multimodal Analytical Platform on a Multiplexed Surface Plasmon Resonance Imaging Chip for Analysis of Extracellular Vesicle Subsets (JOVE, Methods Collections: Novel or Optimized Isolation and Characterization Methods of Extracellular Vesicles, in press).

10:30- 10:45 AM

EV Fingerprinting: Resolving extracellular vesicle heterogeneity using multi-parametric flow cytometry

Ariana K. von Lersner[1] Fabiane Fernandes[2,3] Patricia M. M. Ozawa4 Sierra M. Barone[4] Tatyana Vagner[5] Bong Hwan Sung[4] Mohamed Wehbe[6] Kai Franze[7,8] John T. Wilson[1,2,6] Jonathan M. Irish[1,2,4] Alissa Weaver[1,2,4] Dolores Di Vizio[5] Andries Zijlstra[2,8]

[1]Program in Cancer Biology, Vanderbilt University, Nashville, TN, USA [2]Department of Pathology, Microbiology and Immunology, Vanderbilt University Medical Center, Nashville, TN, USA [3]Institute of Applied Biosciences and Chemistry, Hogeschool Arnhem en Nijmegen University of Applied Sciences [4]Department of Cell and Developmental Biology, Vanderbilt University School of Medicine, Nashville, TN, USA [5]Department of Surgery, Cedars-Sinai Medical Center, Los Angeles, US. [6]Department of Chemical and Biomolecular Engineering, Vanderbilt University, Nashville, TN, USA [7]KNIME GmbH, Konstanz, Germany [8]Department of Research Pathology, Genentech Research and Early Development

Mammalian cells release a heterogeneous array of extracellular vesicles (EVs) that impact human physiology and pathology by contributing to intercellular communication. To resolve EV heterogeneity and define populations of vesicles that are associated with specific biological processes, we developed a method named “EV Fingerprinting” that discerns distinct vesicle populations using dimensional reduction of multi-parametric data collected by quantitative single-EV flow cytometry from EVs stained with a single lipophilic dye. The quantitative detection of EV populations was validated against synthetic standards and orthogonal methods. Analysis of EVs isolated by differential centrifugation demonstrated that EV Fingerprinting can resolve biochemically distinct EV populations. Shifts in spectral emissions associated with lipid composition are the dominant feature that distinguishes EV populations. Unlike large EVs, small EV populations vary greatly in their lipid-associated emission spectra. Consequently, EV Fingerprinting readily identifies EV populations differentially enriched by ultracentrifugation-based isolation methods. Application of EV Fingerprinting to EVs isolated from cell lines with molecular perturbations of EV biogenesis through Rab27a ablation and CD63 overexpression revealed that EV

Fingerprinting reflects the molecular state of a cell. In fact, each perturbation has a selective rather than a uniform impact on the biogenesis of EV populations. Subsequent analysis of human plasma demonstrates the capacity of EV Fingerprinting to resolve EV populations in complex biological samples and detect tumor-derived EV. These studies demonstrate that EV populations can be resolved using single-EV analysis of spectral emissions after staining with lipophilic dyes. We propose that the resulting fingerprint reflects the state of the originating cell and can be leveraged as a biomarker and to generate biological insight.

10:45- 11:00 AM

Femtosecond Laser-fabricated Nanoplasmonic Sensors and Deep Learning Enable Label-free Classification of Extracellular Vesicles

Colin L. Hisey[1,2,6], Mohammadrahim Kazemzadeh[3], Miguel Martinez-Calderon[4], Anastasiia Artuyants[5], Song Y. Paek[6], Varima Narula[7], Ashika Chhana[7], Moimoi Lowe[4], Scott Bolam[8], Claude Aguergaray[4], Jacob Munro[8], Nicola Dalbeth[8], Mark Zhu[8], Peter Xu[3], Sue McGlashan[7], Cherie Blenkiron[2,5], Lawrence W. Chamley[2,6], Neil G.R. Broderick[4]

[1]Department of Biomedical Engineering, The Ohio State University, Columbus, Ohio, USA [2]Hub for Extracellular Vesicle Investigations, University of Auckland, Auckland, New Zealand [3]Department of Mechanical and Mechatronics Engineering, University of Auckland, Auckland, New Zealand [4]Department of Physics, University of Auckland, Auckland, New Zealand [5]Auckland Cancer Society Research Centre, University of Auckland, Auckland, New Zealand [6]Department of Obstetrics and Gynaecology, University of Auckland, Auckland, New Zealand [7]Department of Anatomy and Medical Imaging, University of Auckland, Auckland, New Zealand [8]School of Medicine, University of Auckland, Auckland, New Zealand

Extracellular vesicles (EVs) are diverse lipid-enclosed micro and nanoparticles that are released by all cells and transport lipids, nucleic acids, proteins, and other bioactive contents to other cells via bodily fluids. While EVs have shown immense potential in applications such as liquid biopsies and therapeutics, there is a lack of rapid, label-free, ultrasensitive, and non-destructive techniques which can simultaneously provide information regarding the various biochemical contents within an EV sample. One potential solution involves the use of surface-enhanced Raman spectroscopy (SERS) to biochemically fingerprinting EVs in combination with machine learning algorithms to both preprocess and classify the otherwise complex and information-rich spectra. Over the past few years, we have developed several complimentary technologies including femtosecond laser-fabricated nanoplasmonic substrates which are both scalable and inexpensive, deep learning-based preprocessing architectures which can rapidly perform unbiased baseline correction and denoising on the acquired spectra, and autoencoder-inspired classifiers which can separate EVs based on their latent spectral features while also providing interpretable information related to the classification. We will present our successful implementation of these technologies in several distinct EV applications, including the characterization and classification of EVs from healthy and preeclamptic placental tissue explant cultures, EVs from the synovial fluid of obese and non-obese patients undergoing knee replacement surgery, as well as a quantitative detection workflow which can determine the relative amounts of exogenous EVs from fetal bovine serum within mixtures of EVs produced from breast cancer EV bioreactors. We are rapidly developing this suite of user-friendly technologies with the aim of making them available to other EV researchers who may not have expertise in the field.

11:00- 11:15 AM

"EV painting" as novel synthetic biology approach to identify EV target cells in vivo

Ferdinando Pucci[1] Natalie Claudio[1] Zihan Guo[1] Dhanya Nambiar[2]

1Department of Cell, Developmental & Cancer Biology; Department of Otolaryngology-Head and Neck Surgery; Program in Biomedical Sciences; Oregon Health and Science University, Portland, Oregon
2Department of Radiation Oncology - Radiation Therapy, Stanford, San Francisco, California

Emerging evidence suggests that tumor-derived extracellular vesicles (tEVs) can influence immune cell behavior both locally within the tumor microenvironment and remotely by accessing the lymphatic and systemic circulation. The in vivo effects of such interactions remain largely unappreciated. Investigation of tEVs' roles in tumor-host communication has been hampered by the need to isolate them before intravenous reinfusion in animals, which can introduce biases such as homogenization of tEV diversity, judgement of amount to reinfuse and assumptions on delivery mode (eg. blood vs lymph). To circumvent these issues, we and others pioneered the use of genetically engineered tumor cells to express membrane-bound reporters, which allow to track untouched, endogenously released tEVs after implantation of parental tumor cells. Still, the amount of reporter proteins that tEVs can carry is very small and the identification of immune cells targeted by tEVs remains a major challenge in the field. Novel approaches able to clearly identify tEV-targeted immune cells will allow to profile the signaling pathways that tEVs employs to affect immune cell behavior. To this end, we have engineered tEVs to display an enzyme (Sortase-A) able to catalytically label tEV binding partners. Sortase A (SrtA), a bacterial transpeptidase, catalyzes the transfer of reporter proteins on the much bigger surface of tEV-binding cells, thereby allowing to greatly increase detection sensitivity. SrtA catalyzes the formation of a peptide bond between a consensus peptide (LPETG) and an N-terminal glycine of nearby proteins, which are abundantly present in both human and mouse cells. As compared to indirect labeling of EV-binding cells (e.g. CD63-GFP fusion), SrtA-based approach shows 1-2 log increase in sensitivity. In vivo testing of this "EV painting" approach highlighted how tEVs size and lymph node anatomy intersect to allow only specific immune cells to interact with tEVs. One of these immune subsets was unexpectedly found in pre-metastatic sites, suggesting a novel immune mechanism of metastasis. We are now adapting this technology to barcode tEV-experienced immune cells for single cell profiling.

11:15- 11:30 AM

The development of an EV and cargo tracking mouse for assessing vesicle trafficking among tissues and transfer from dam to pup by milk

Janos Zempleni[1] Wei Zhao[1] Haichuan Wang[1] Mengna Xia[1] Hideaki Moriyama[2] Masato Ohtsuka[3]

[1]Dept. of Nutrition and Health Sciences, [2]School of Biological Sciences, U. Nebraska-Lincoln, Lincoln, NE, [3]Dept. of Molecular Life Science, Tokai University, Kanagawa, Japan

Introduction: Availability of a mouse for tracking EV and cargo trafficking among tissues accelerates the rate of discovery in EV-dependent communication. Goal: Develop an EV and Cargo Tracking (ECT) mouse to assess the origin, destination, and cargo of EVs. Aims: 1) Develop and validate an ECT mouse. 2) Use the ECT mouse to assess the transfer of EVs from dam to pups by milk and EV accumulation in regions of the neonate brain. Methods: The ECT plasmid encodes a of CD63-eGFP fusion protein, flanked by two loxP sites (ORF-1). A second ORF, coding for a CD63/near-infrared protein (iRFP) fusion protein, follows

downstream of ORF-1. A transmembrane domain is fused to the C-terminus of the iRFP to position the C-terminus on the outer EV surface where it is fused with a second iRFP. In the presence of Cre, the CD63/eGFP/Stop insert is removed, and the mice switch from expressing eGFP-labeled EVs to iRFP-labeled EVs, including a surface iRFP for collecting EVs by affinity chromatography. In studies of milk EVs, wild-type (WT) pups were fostered to ECT dams or WT controls and distribution in the brain was assessed by serial two-photon tomography. Results: In the absence of Cre, ECT mice expressed eGFP-labeled EVs, whereas the progeny of mice mated with Cre mice expressed iRFP-labeled EVs. Fluorescent proteins localized to small EVs but not to other complexes secreted by HEK-293 cells transfected with the ECT plasmid. The EVs were size 131 ± 49 nm, stained positive for CD9, Alix and TSG101, stained negative for nuclear material, and were captured by anti-CD63. When WT pups were nursed by ECT dams for 17 days, EVs accumulated in the various regions of the brain. Conclusions: We have developed a mouse that permits tracking CD63-positive EVs and their cargos. CD63-positive EVs are bioavailable and accumulate in the brain in suckling mice. Funding: NIFA, NIH, SynGAP Research Fund, USDA. JZ is as consultant for PureTech Health.

2:00- 2:15 PM

miREV an online database and meta-analysis tool to identify stable reference miRNA signatures -- A step towards standardization in EV quantitative transcriptomics

Michael W. Pfaffl[1], Alex Hildebrandt[1], Esther N. Nolte-'t Hoen[2], Benedikt Kirchner[1]

[1]Division on Animal Physiology and Immunology, Technical University of Munich, Freising, Germany; [2]Department of Biochemistry and Cell Biology, Utrecht University, Utrecht, The Netherlands.

To verify transcriptomic results in EV RNA-Seq studies, biomarker transcripts are commonly validated using RT-qPCR. The resulting biomarker signature should be as accurate as possible; hence a proper normalization procedure between individuals and groups by stable reference transcripts is required, which is still elusive in the EV transcriptomics field. Different treatments, studies or experimental setups will likely yield distinct candidate reference miRNAs, which hampers comparability across studies. To setup a meta-analysis tool to identify these stable reference miRNA signatures and collect it in an online database, we established miREV. We collected in-house and publicly available metadata on pre-analytical variables, EV isolation, RNA isolation, and small RNA-Seq data. Today the database focuses on human blood derived EVs and includes 9 diseases, 3 isolation methods from both serum and plasma. All datasets comprising a total of 654 samples were processed by a standardized data analysis pipeline to account for a variety of experimental setups. Robust filter steps to remove data of poor quality, as well as six different normalization methods (total count, median, quantile, upper quartile, trimmed mean of M-values and median of expression ratios) and three stability algorithms (BestKeeper, geNorm, NormFinder) for finding robust reference transcripts were implemented. Results can be further refined by EV isolation method, disease or treatment context to match experimental setups as close as possible to individual users. Suggested reference candidate lists are visualized to easily facilitate comparisons and to detect influences of different experimental procedures or the disease context. The obtained suggestions for a stable reference miRNAs are made available in an easy-to-use web tool https://www.physio.wzw.tum.de/mirev/ miREV represents a useful tool for scientists to find suitable reference transcripts for their own EV experiments without the need to priorly run RNA-Seq. The inclusion of additional data sets in the near future that span a variety of patient populations, sample or tissue types, or EV isolation methods will boost flexibility and increase the statistical validity and robustness of suggested reference transcripts for all kinds of EV quantitative transcriptomics.

2:15- 2:45 PM

Metrological flow cytometry for traceable determination of refractive index and size of single extracellular vesicles and reference materials

Martine Kuiper[1,2,3,4], Richard Koops[2], Rienk Nieuwland[1,3], Edwin van der Pol[1,3,4,5,6]

[1]Laboratory Experimental Clinical Chemistry; Amsterdam University Medical Centers, Location AMC, University of Amsterdam, Amsterdam, the Netherlands; [2]VSL, National Metrology Institute, Delft, the Netherlands; [3]Vesicle Observation Center; Amsterdam University Medical Centers, Location AMC, University of Amsterdam, Amsterdam, the Netherlands; [4]Biomedical Engineering and Physics; Amsterdam University Medical Centers, Location AMC, University of Amsterdam, Amsterdam, the Netherlands [5]Amsterdam Cardiovascular Sciences, Atherosclerosis and Ischemic Syndromes, Amsterdam, the Netherlands [6]Cancer Center Amsterdam, Imaging and Biomarkers, Amsterdam, the Netherlands

Extracellular vesicles (EVs) have high potential as biomarkers of disease. EVs are commonly measured by flow cytometers, which measure both fluorescence and light scattering of single EVs in fluids. Using Mie theory, the arbitrary units of light scattering signals can be related to the diameter of EVs when the refractive index (RI) is known. A method to traceably determine the RI of nanoparticles including a well-defined uncertainty, however, is lacking. Therefore, we are developing a metrological flow cytometer (mFCM) to traceably determine the RI, size, and number concentration of EVs in a fluid. The mFCM, constructed by the Dutch National Metrology Institute, has six technical advantages over clinical state-of-the-art flow cytometers. The mFCM (i) utilizes a Laguerre-Gauss beam to illuminate particles, making a blocker bar unnecessary, (ii) simultaneously measures the angle-dependent light scattering of single particles in back-, forward-, and side-scattering directions, (iii) relates signals to theory using a convolutional neural network to determine the particle properties, (iv) has an interrogation volume 500-fold smaller than clinical flow cytometers, thereby increasing the probability of real single particle detection, (v) has an acquisition time 2,500-fold longer than clinical flow cytometers, thereby improving the measurement precision, and (vi) has a calibrated syringe pump with a relative measurement uncertainty of ±2.75e-3. The mFCM will be used to perform the first traceable RI, size, and number concentration measurements of EVs and reference materials, which can be used to calibrate clinical flow cytometers. We anticipate that the mFCM is a prerequisite for reproducible and comparable EV concentration measurements by flow cytometry, and will become a benchmark technique for traceable characterization of nanoparticles in suspension.

2:45 - 3:00 PM

French Interlaboratory study for standardization in EVs metrology

D. Aubert, K. Aubertin, A. Audfray, S. Audonnet, S. Banzet, M. Berger A. Berquand, A. Bianchi, W. Boireau, S. Bosch, B. Brassart, S. Brassart, C. Butler, P. Defrenaix, P. Ducoroy, T. Fourniols, F. Gazeau, J. Gobbo, Z. Krupova, R. Lacroix, Q. Lubart, E. Madec, D. Mai, P. Mauduit, L-E. Monfoulet, M. Morani, M. Morille, C. Neri, J. Peltzer, C. Pinte, C. Ramassamy, G. Raizada, R. Rivet, A. Rouleau, C. Schietroma, M. Taverna, M-L. Trouillas, A. Van Der Heyden, E. Velot, J. Volatron and C. Elie-Caille

In this study, launched in April 2022, almost 20 labs, and several companies, are analyzing one single sample, produced by Everzom (MSC derived EVs), under several subgroups working specifically with NTA, TRPS, FC, nanoscopy or AFM & EM microscopies, for the purpose of qualifying EVs size. The main idea is to use the same parameters in all the different labs using the same technics, to appreciate the variability between labs operating on the same method. We have been also evaluating the impact of the instruments age (= number of hours of use for laser in NTA for example). Thus, the study enables to highlight specific and important parameters to choose, for each technic, to measure correctly the EVs size. In microscopy approaches, our study reveals the extent of the preparation steps, and also of the substrate (its roughness, structuration and biointerface) on EV size and shape measurements. Altogether, this study consists in obtaining and comparing the results of EV metrology obtained by different approaches, one the same EVs sample, produced in the context of bioproduction for biotherapies. Each academic and industrial partners realized a triplicate of experiments with his specific technic. All the data were saved following a devoted nomenclature, and stored in a data base that was specifically built for this project ; the company BIOMANEO (Dijon, France) which built the data base, also processed the data in terms of analysis, comparison and statistics. The end of this study is planified for next December 2022. This study should enable to propose to the EV community a particular combination of technics - with specific working conditions and parameters - to qualify, in a precise and fair way, EVs metrology, in the context of EVs bioproduction from cell lines and their use in biotherapy. The short term perspective consists, with an expanded consortium, in determining the best technics or combination of methods, to qualify in size and also in morphomechanical properties, EVs in a clinical context (directly from biofluids, like human plasma).

Poster Presentations

Wednesday, November 16

5:00- 6:30pm

Poster Session A

P01	Arturs	Abols	Mesenchymal stem cell-derived extracellular vesicles as a cis-platin carriers in PDMS-free lung cancer-on-a-chip model
P02	Farrukh	Aqil	Extracellular vesicle-based nano 'platform' technology for RNA and DNA delivery
P03	Jessie	Arce	NanoFlow Repository: a data-sharing resource for EV research
P04	Zbigniev	Balion	Effect of extracellular vesicles derived from stem cells on osteoblasts in medication-related osteonecrosis in vitro model
P05	Felix	Baumann	Catching diagnosis relevant cancer small extracellular vesicles (sEV) with nanoparticles: Optimization of a two-step orthogonal recognition approach
P06	Madhusudhan	Bobbili	CD81-based combinatorial library for selecting recombinant binders to cell surface proteins: To enhance the ability of their targeted delivery
P07	Jonathan	Burnie	Adapting a novel platform for screening cellular proteins on the surface of virus particles with nanoscale flow cytometry
P08	Kaiping	Burrows	Comparison of neuronal-enriched extracellular vesicles using different extracellular vesicles isolation methods
P09	Sara	Cavallaro	Use of signal amplification strategies for fluorescent tagging of extracellular vesicles isolated from patients with glioblastoma
P10	Sarah	Cox-Vazquez	A New Class of Water-soluble, Amphiphilic, Membrane Spanning Dyes and Their Use in Extracellular Vesicle Research
P11	Vanessa	Dartora	Modulation of the secretome profile of endothelial progenitor cells with bioactive glycosaminoglycan materials to improve pressure ulcer healing
P12	Aslan (Mehdi)	Dehghani	Improvements to the Quantification of Nanoparticles and Extracellular Vesicles
P13	Magdalena	Dlugolecka	Characterization of extracellular vesicles from placenta stem cells
P14	Brian	Dobosh	Development of a designer EV tookit (DEVKIT) for the creation of fully customized EVs
P15	Ahmed	Elsherbini	A novel method for isolation of Extracellular Vesicles (EVs) from brain tissues using gentle proteolytic dissociation and membrane affinity capture
P16	Leandra	Figueroa-Hall	Comparison of next-generation sequencing data analysis on astrocyte-enriched extracellular vesicle microRNA using different platforms
P17	Tal	Gilboa	Isolation of Extracellular Vesicles (EVs) by removal of both free proteins and lipoproteins, and accurate measurement of intra- EVs proteins
P18	Ruediger	Gross	Detection of EV-exposed phosphatidylserine extends the capabilities of bead-based flow cytometry
P19	Ramesh	Gupta	Isolation of small extracellular vesicles from bovine colostrum powder using a combination of techniques
P20	Christina	Guzzo	A virologist's toolbox for studying virion-incorporated host proteins.
P21	Yuki	Harada	Evaluation of Storage Buffer for Efficient Preservation of Bioactive Engineered Extracellular Vesicles
P22	Ryan	Hogans	Multi Omics Profiling of Endothelial Cell Extracellular Vesicles Elucidates Roles in Senescence Associated Vascular Dysfunction

P23	Elham	Hosseini-Beheshti	High-throughput Astrocyte and Neuron derived small EV sorting using (iZExoSub) inertial microfluidics: proof of concept application in the diagnosis and monitoring of neurodegenerative disease
P24	Brianna	Huszti	Production and Characterization of Extracellular Vesicle Reference Samples for Rigorous and Reproducible EV Assays.
P25	Diana	Kitka	Orthogonal application of traditional and innovative technologies to purify cancer-derived large extracellular vesicles
P26	Natalia	Krawczynska	27-hydroxycholesterol neutrophils small extracellular vesicles promote breast cancer progression
P27	Martine	Kuiper	Refractive index determination of liquids with the lowest uncertainty worldwide to improve standardization of EV flow cytometry
P28	Michael	LeClaire	Lab Scale Exosome Isolation for Therapeutic Development
P29	Dasol	Lee	Size-based sorting of tumor EVs using viscoelastic and secondary co-flows in microfluidic device
P30	Biao	Lu	A Novel Integrated Software for Streamlined Design and Production of Exosome-based Nanovaccines
P31	Evelyn	Luciani	A Microfluidic Assay for the Isolation of SARS-CoV-2 and Cell-Specific Extracellular Vesicles in Plasma
P32	Setty	Magana	Multiparametric characterization of bulk and single EVs from paired human adipose tissue and plasma—towards the development of an adiposity liquid biopsy
P33	David	Marciano	Investigating the Effects of Laminar Shear Stress on Endothelial Cell Derived EV
P34	Thomas	Maslanik	MIFlowCyt-EV reporting of single vesicle flow cytometry methods and results

Thursday, November 17

5:00- 6:30pm

Poster Session B

OLD Poster #	New Poster #	First Name	Last Name	Abstract Title
P35	P01	Rachel	Mizenko	Membrane dilution: a new approach for EV-liposome hybrids to improve drug delivery
P36	P02	Eqbal	Mohamadi	Isolation Of Aloe vera extracellular vesicles; low-cost fast method
P37	P03	Sujata	Mohanty	A cost-effective lyophilized exosome (LyoExo) embellished implantable biomaterial construct for sustained release & regeneration and off-the-shelf utility
P38	P04	Jisook	Moon	Placenta EV-associated regulation of OCT4/SOX2, a key mechanism in delaying aging.
P39	P05	Amber	Murray	Scalable, chip-based method for high-yield, high-purity EV isolation
P40	P06	Gi-hoon	Nam	Maximizing Therapeutic Proteins on Exosomes
P41	P07	Truc	Nguyen	Multi-Parametric Integrated Molecular Detection of Various SARS-CoV-2 Strains from Biofluids by Adapting Single Extracellular Vesicle Characterization Technologies
P42	P08	John	Nolan	Quantitative analysis of molecular cargo transfer from tumor cells to EVs
P43	P09	Juhee	Park	ExoDisc: A Centrifugal Tangential Flow Filtration for Extracellular Vesicle Separation from Clinical Samples of Cancer Patients
P44	P10	Ben	Peacock	Specific labelling and identification of EVs within complex particle isolations
P45	P11	Michael W.	Pfaffl	Comprehensive small RNA Seq data analysis computational pipeline -- Obtaining valid biomarker signatures from EVs made easy
P46	P12	Desmond	Pink	Multi-angle flow cytometry illustrates the refractive index complexity of commonly evaluated biological samples for extracellular vesicle analysis
P47	P13	Desmond	Pink	Qualifying EV Technologies for Regulated and non-Regulated Research
P48	P14	Bonita	Powell	Ex-vivo analysis of extracellular vesicle interactions with immune cells in blood of primates
P49	P15	Beata	Pyrzynska	Beneficial Effects of Salinomycin on Immunotherapy of Lymphoma
P50	P16	Daniel	Rabe	Microfluidic capture of tumor and macrophage extracellular vesicles (EVs) in triple-negative breast cancer (TNBC)
P51	P17	Truc	Nguyen	An Immunogold Single Extracellular Vesicular RNA and Protein (AuSERP) Biochip to Predict Responses to Immunotherapy in Non-Small Cell Lung Cancer Patients
P52	P18	Daniel	Ruiz	Characterization of the impact of combinatorial anti-PD-1 and TLR-8 agonism in Head and Neck Squamous Cell

				Carcinoma using single cell RNA-seq, Extracellular Vesicles and Multiplex Staining Imaging Techniques
P53	P19	Magdalena	Schimke	Characterisation of extracellular vesicles from the placenta Stem cells
P54	P20	Satyajyoti	Senapati	Charged Based Fractionation and Profiling of Nanocarriers and Their Molecular Cargoes
P55	P21	Taylon	Silva	Integrative analysis of whole proteome and transcriptome of cancer-derived extracellular vesicles confirms results from single EV RNA-Seq
P56	P22	Taehwang	Son	Single extracellular vesicle detection and molecular profiling using plasmon-enhanced fluorescence detection
P57	P23	INO	SONG	Therapeutic effects of the highly purified extracellular vesicles from stem cells for temporomandibular joint repair
P58	P24	Karolina	Soroczyńska	Searching for novel biomarkers of endometriosis using imaging flow cytometry analysis of EVs derived from biological fluids of endometriosis patients
P59	P25	Elias	Spiliotopoulos	Extracellular Vesicles' Short and Long RNA Sequencing Using Low Plasma Input in Human and Non-Human Primates
P60	P26	Siddharth	Srivastava	Au Nanopyramid-based SERS Platform for Single Vesicle Biochemical Characterization
P61	P27	Li	sun	MagPEG: a complete extracellular vesicle isolation/analysis solution
P62	P28	Vijaya	Sunkara	ExoPRISM: A Rapid, Scalable Method for Extracellular Vesicle Separation
P63	P29	Rucha	Trivedi	Molecular contribution of Annexin A2 in triple negatived breast cancer metastasis via tumor-derived extracellular vesicles
P64	P30	Thupten	Tsering	EV-ADD, a database for EV associated DNA in human liquid biopsy samples
P65	P31	Sara	Veiga	Optimizing single EV imaging inside a microfluidic device
P66	P32	Ariana	von Lersner	EV Fingerprinting resolves the selective alteration of small EV secretion in response to loss of the GTPase Rab27a
P67	P33	Ceming	Wang	A Next-Generation High-Efficiency Extracellular Vesicle (EV) Isolation Platform, NanoEX, for Diagnostics and Therapeutics
P68	P34	Gina	Wang	Single-molecule imaging of circulating extracellular vesicles for disease diagnosis
P69	P35	Saigopalakrishna	Yerneni	Radioiodination of extravesicular surface constituents to study the biocorona, cell trafficking and storage stability of extracellular vesicles
P70	P36	Zhengrong	Zhang	A comparative analysis of different human brain-derived extracellular vesicle isolation methods