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Oral Presentations

Thursday, November 21

8:35 AM - 8:50 AM

Acoustofluidic technologies for the manipulation of cells, extracellular vesicles, and other bioparticles

Tony Jun Huang, Pratt School of Engineering, Duke University

The use of sound has a long history in medicine. As acoustic technology has advanced, so too has our ability to "listen" to the body and better understand underlying pathologies. The stethoscope allowed doctors to gauge the health of the heart; the ultrasound imaging revolutionized the field of biomedical imaging and enabled doctors to diagnose a range of conditions in the fields of obstetrics, emergency medicine, cardiology, and pulmonology. In the last decade, a new frontier in biomedical acoustic technologies has emerged, termed acoustofluidics, which joins cutting-edge innovations in acoustics with micro- and nano- scale fluid mechanics. Advances in acoustofluidics have enabled unprecedented abilities in the early detection of cancer, the non-invasive monitoring of prenatal health, the diagnoses of traumatic brain injury and neurodegenerative diseases, and have also been applied to develop improved therapeutic approaches for transfusions and immunotherapies. In this talk, I summarize our lab's recent progress in this exciting field and highlight the versatility of acoustofluidic tools for biomedical applications through many unique examples, ranging from the development of high-purity, high-yield methods for the separation of circulating biomarkers such as small extracellular vesicles (sEVs) and circulating tumor cells (CTCs), to our newly developed harmonic acoustics for a non-contact, dynamic, selective (HANDS) particle manipulation platform, which enables the reversible assembly and disassembly of cells. These acoustofluidic devices can precisely manipulate objects across 7 orders of magnitude (from a few nanometers to a few centimeters). Thanks to these favorable attributes (e.g., versatility, precision, and biocompatibility), acoustofluidic devices harbor enormous potential in becoming a leading technology for a broad range of applications, playing a critical role for translating innovations in technology into advances in biology and medicine.

8:50 AM - 9:05 AM

Label-Free Microelectronic Lab-on-a-Chip Device for Purification and Characterization of Small Extracellular Vesicles

Leyla Esfandiari, University of Cincinnati

Small extracellular vesicles (sEVs), lipid-bilayer delimited nanoparticles naturally secreted by all cells, have attracted substantial interest as indispensable biomarkers spanning a diverse range of diseases. The isolation of sEVs with high efficiency, maintaining optimal yield and purity, is pivotal for unlocking their full potential in diagnostic, prognostic, and therapeutic applications. However, the intricate nature of clinical samples and the heterogeneous physicochemical properties of extracellular vesicles (EVs) present formidable challenges in their accurate isolation and characterization, particularly when derived from complex body fluids. In response to these challenges, our research has led to the development of a cutting-edge and patented electrokinetic-based microchip. This microchip stands as a simple yet powerful solution, specifically designed for the rapid and label-free purification of sEVs from various

body fluids and cell culture medium. Leveraging a significantly low electric field, this innovative device streamlines the isolation process, ensuring both speed and efficiency. Notably, the microchip goes beyond mere isolation. It is tailored with a sophisticated sensing module that facilitates the detailed characterization of sEVs. This characterization is achieved by measuring the impedance of sEVs, providing insights into their dielectric properties. This multifaceted approach to sEV analysis enhances our capacity to understand and utilize these vesicles as crucial components in liquid biopsy procedures. This novel technology holds the potential to revolutionize diagnostic precision and drive therapeutic advancements in clinical practice, especially as we delve deeper into unraveling the complexities of sEVs.

9:05 AM - 9:20 AM

Harnessing Small Extracellular Vesicles Cargo to overexpress tumor suppressive microRNAs to Inhibit Breast Cancer Metastasis

Tyler Offenbacher[1], Russell O. Bainer[2], Jiyoung Lee[3,4], Casey Frankenberger[5], Irina Sbornova[2], Ayoolwakiitan Oluwafemi[2], Keyata Thompson[6], Jiajia Gu[6], Min Yu[6], Stuart S. Martin[6], Marsha R. Rosner[1], and Nykia D. Walker[1,3,6]

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Triple-negative breast cancer (TNBC) is an aggressive breast cancer subtype with limited effective treatments. Tumor-derived extracellular vesicles (TD-EVs) play a crucial role in tumor progression, but their potential to reprogram non-malignant stromal cells remains largely unexplored. Using TNBC xenograft mouse models coupled with species-specific RNA sequencing (RNA-seq) analysis, we identified a subset of TD-EV- mediated genes, including MMP13, TGF-β, SERPINE1, and VIM, which are involved in extracellular matrix remodeling, cell invasion, and bone metastasis. Functional studies revealed that MMP13-enriched TD-EVs promote fibroblast reprogramming and cell invasion by downregulating the tumor suppressor Let-7 miRs and upregulating onco- miRs.BACH1, a transcription factor associated with cell invasion and metastasis, is negatively regulated by Let-7 miRs. We demonstrated that BACH1 overexpression in TNBC cells downregulates Let-7c and Let-7g, resulting in a three-fold increase in invasive properties compared to TD-EV knockdown cells. Conversely, Raf kinase inhibitory protein (RKIP) inhibits metastasis by regulating Let-7 expression and indirectly suppressing BACH1. Our findings indicate that targeting MMP13 and BACH1 with Let-7 miR mimics presents a promising therapeutic strategy to inhibit TNBC cell invasion, potentially impeding cancer spread and shifting cells toward a proliferative state that increases their susceptibility to chemotherapy. Future studies will focus on engineering TD-EVs with Let-7c and Let-7g via nanocarriers or genetic modifications of exosome-producing cells. This approach not only allows for targeted delivery of Let-7 miRs to metastatic sites but may also overcome systemic delivery challenges, utilizing the natural targeting capabilities of EVs to effectively inhibit metastasis in TNBC patients.

9:20 AM - 9:35 AM

Light-induced Extracellular Vesicle and Particle Adsorption

Colin Hisey[1], Xilal Rima[1], Jacob Doon-Ralls[1], Chiranth Nagaraj[1], Sophia Mayone[1], Kim T. Nguyen[1], Sydney Wiggins[1], Kalpana Deepra Priya Dorayappan[1], Karuppaiyah Selvendiran[1], James N. Higginbotham[2], Oleg Tutanov[2], Jeffrey L. Franklin[2], Robert J. Coffey[2], David Wood[1], Chunyu Hu[1], Divya Patel[1], Andre Palmer[1], Derek Hansford[1], Eduardo Reátegui[1]

[1]The Ohio State University; [2]Vanderbilt University

The role of extracellular vesicles (EVs) in human health has garnered considerable attention over the past two decades. However, while several types of EVs are known to interact dynamically with the extracellular matrix (ECM) and there is great potential value in producing high-fidelity EV micropatterns, there are currently no label-free, high-resolution, and tunable platform technologies with this capability. We introduce Light-induced Extracellular Vesicle Adsorption (LEVA) as a versatile EV micropatterning technique that should rapidly advance the study of ECM- and surface-bound EVs. LEVA occurs when UV illumination is selectively applied to PLL/PEG-functionalized surfaces using a digital micromirror device (DMD), creating microdomains on the surface with tunable EV binding affinity based on the grayscale values of input templates. LEVA's versatility is demonstrated using commercial GFP-EV standards, E. coli outer membrane vesicles (OMVs), DiFi exomeres, and U-87 MG EVs to create micropatterns of the EVs on surfaces with different geometric shapes and gradients. Following initial optimization, several applications were tested and EVs were characterized according to MISEV guidelines, including NTA, TEM, zeta potential, and immunoblotting. Time-lapse TIRFM and COMSOL simulations demonstrated slower adsorption kinetics of large EVs compared to small EVs due to a combination of Brownian motion and charge. LEVA enabled the characterization of single EVs by TIRFM colocalization of fluorescent probes, observation of U-87 MG single cell migration behavior on "1D" U-87 MG migrasome-mimetic trails, and characterization of OMV micropattern geometry effect on localized peripheral blood-derived neutrophil swarming. LEVA's scalability, high-resolution, speed, and versatility will enable rapid advancements in the study of ECM- and surface-bound EVs and should encourage researchers from many disciplines to create novel assays for limitless future EV applications.

10:30 AM - 10:45 AM

Isolation, characterization, and therapeutic application of matrix-bound nanovesicles, a type of extracellular vesicle derived from the extracellular matrix

Marley Dewey, PhD, University of California Santa Barbara, Santa Barbara, CA

The extracellular matrix is a critical component of all tissues and is required for tissue repair. Discoveries of the elements within the extracellular matrix have led to advances in our understanding and development of therapeutics for wound repair. Of these elements are matrix-bound nanovesicles (MBVs), a newly discovered type of extracellular vesicle tightly embedded within the extracellular matrix. MBV can be found in mammalian tissues including brain, heart, bladder, muscle, among others, as well as the matrices produced by other organisms such as fungi (biofilms). MBVs are distinct from other extracellular vesicle types and have different cargo, surface markers, and lipid compositions. The

isolation, characterization, and therapeutic potential of MBVs will be discussed as it relates to other extracellular vesicle populations. MBV isolation from both in vivo tissue samples and in vitro ECM will be discussed. MBVs are characterized by a similar size range to exosomes (50-200 nm), vesicle-like morphological features (TEM imaging), different enrichment of surface markers common to other extracellular vesicle populations, and cargo contents (microRNA, proteins) related to organ and tissue development. These results are compared to extracellular vesicles derived from the liquid (blood, cell culture medium, etc.) and matrix vesicles found in bone and calcifying cells. Encouraging therapeutic applications of MBVs include the ability to regenerate damaged nerves after optic nerve crush injuries and the ability of MBV to ameliorate pristane-induced chronic arthritis. MBVs are promising extracellular vesicles but there remain many unknown questions of their purpose and biogenesis as it relates to the extracellular matrix, answers to which could revolutionize how we combat disease and wound repair. As a new lab, I will discuss a preview of work in my laboratory, in the characterization and use of mesenchymal stem-cell derived MBV to answer these remaining questions.

10:45 AM - 11:00 AM

Label-free sizing and multiplexed protein detection on single extracellular vesicles

Andreas Wallucks[1], Philippe DeCorwin-Martin[1], Lucile Alexandre[1,2], Hugues Martin[1], Yonatan Morocz[1] Andy Ng[1], and David Juncker[1]

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Just as single-cell RNA sequencing has revolutionized the study of cellular diversity, high multiplex single EV proteomics could transform our understanding of EV heterogeneity by mapping the EV proteomic landscape, identifying subpopulations, and establishing EV-based diagnostics. Current single EV proteomic technologies, such as flow cytometry, fall short due to high detection thresholds, limited protein multiplexing, or both. Here we introduce EV-ID, a platform with a detection limit around 35 nm, capable of detecting dozens of proteins simultaneously in tens of thousands of single EVs from a 10 ÂµL sample, in a fully automated, scalable well-plate format. EVs from cell supernatant or unpurified plasma are immobilized on off-the-shelf glass substrates functionalized in-house forming both universal capture surfaces for unbiased EV capture, and affinity surfaces for the enrichment of subpopulations. Captured EVs are counted and sized using previously introduced EV size photometry (SP). We perform SP by label-free interferometric scattering microscopy (iSCAT) using a widefield epifluorescence microscope with a 50:50 beamsplitter. EV protein expression is mapped via DNA exchange imaging, where EVs are immunolabeled with a panel of DNA-barcoded antibodies. Cyclic fluorescence imaging allows rapid detection of each barcode with labelling and erasure performed in under 5 minutes each. The data acquisition is fully automated using a robotic liquid handling system on custom wellplates, managing cyclic imaging of multiple samples in parallel. We showcase EV-ID by measuring a panel of 20 proteins on over 10,000 EVs from HT29, HEK293T, and MDA-MB-231 cells, generating high-dimensional maps of protein distributions that reveal universal and cell-specific co-expression patterns. EV-ID fills a critical gap in single EV analysis by enabling highly sensitive, multiparametric, and high-throughput data acquisition, advancing fundamental EV biology and EV-based diagnostics.

11:00 AM - 11:15 AM

Extracellular vesicles carry transcriptional 'dark matter' revealing tissue-specific information

Navneet Dogra, Icahn School of Medicine

From eukaryotes to prokaryotes, all cells secrete extracellular vesicles (EVs) as part of their regular homeostasis, intercellular communication, and cargo disposal. Accumulating evidence suggests that small EVs carry functional small RNAs, potentially serving as extracellular messengers and liquid-biopsy markers. Yet, the complete transcriptomic landscape of EV-associated small RNAs during disease progression is poorly delineated due to critical limitations including the protocols used for sequencing, suboptimal alignment of short reads (20–50 nt), and uncharacterized genome annotations—often denoted as the 'dark matter' of the genome. In this study, we investigate the EV-associated small unannotated RNAs that arise from endogenous genes and are part of the genomic 'dark matter', which may play a key emerging role in regulating gene expression and translational mechanisms. To address this, we created a distinct small RNAseq dataset from human prostate cancer & benign tissues, and EVs derived from blood (pre- & post-prostatectomy), urine, and human prostate carcinoma epithelial cell line. We then developed an unsupervised data-based bioinformatic pipeline that recognizes biologically relevant transcriptional signals irrespective of their genomic annotation. Using this approach, we discovered distinct EV-RNA expression patterns emerging from the un-annotated genomic regions (UGRs) of the transcriptomes associated with tissue-specific phenotypes. We have named these novel EV-associated small RNAs as 'EV-UGRs' or "EV-dark matter". Here, we demonstrate that EV-UGR gene expressions are downregulated by ~100 fold (FDR< 0.05) in the circulating serum EVs from aggressive prostate cancer subjects. Remarkably, these EV-UGRs expression signatures were regained (upregulated) after radical prostatectomy in the same follow-up patients. Although further validation in randomized clinical trials is required, this new class of EV-RNAs hold promise in liquid-biopsy.

Friday, November 22

9:15 AM - 9:30 AM

Leveraging Similarities between Vesicles and Viruses to Develop Novel Diagnostic Tools

Kim Truc Nguyen[1], Xilal Rima[1], Eduardo Reátegui[1]

[1]Department of Chemical and Biomolecular Engineering The Ohio State University

Liquid biopsies, a novel approach involving the sampling and analysis of tumor-derived content from a blood draw, are gaining rapid interest in the laboratory and clinic. Their significant advantages over traditional tissue analysis, such as frequent sampling and less invasiveness, make them a promising avenue for cancer and infectious disease detection. One potential tool for developing liquid biopsy assays is extracellular vesicles (EVs). These tiny lipid nanoparticles, continuously shed from tumor cells and found in various biofluids, can serve as a surrogate for tumor cells due to their similar proteins, mRNAs, and miRNAs. This presentation will delve into the description of different technologies for in-situ simultaneous protein and RNA profiling of subpopulations of EVs and lipoproteins at the single particle level, using total internal reflection fluorescence (TIRF) microscopy. We have recently extended

our EV expertise to a single virus particle characterization technology that surpasses standard antigen and molecular detection of SARS-CoV-2. The translational potential of our technologies will be demonstrated through proof-of-concept experiments conducted with different biofluids from cancer and COVID-19 patients.

9:30 AM - 9:45 AM

Graphene-based field-effect transistor biosensors for sensitive detection of exosomes

Guojun Zhang, Hubei University of Chinese Medicine

Exosomes, which are subcellular nanovesicles secreted by almost all cells to extracelluar surroundings, have been regarded as one of the main biomarkers of liquid biopsy. Developing a high-performance exosome detection platform is crucial for the early diagnosis of various diseases, especially cancer. Graphene-based field-effect transistor (GFET), as an electronic biosensor with infinite bright application prospect developed in recent years, holds the following unparalleled advantages, such as high sensitivity, fast analysis speed, label-free detection, etc. Hence, GFET is expected to become a powerful point-of-care tool for diagnosing exosomes-related diseases in future. Herein, the GFET biosensors were constructed and successfully used for the label-free detection of exosomes, with a detection limit of 33 particles/μL. Based on this, an integrated exosome detection system was further established by combining a magnetic separation with the GFET detection, which can accurately differentiate between pancreatic cancer patients and healthy people. In addition, an AuNPs-modified GFET sensor was also developed for the highly sensitive detection of the neuron-derived exosomal Aβ42, demonstrating good diagnostic ability for Alzheimer's disease (AD) patients.

10:30 AM - 10:45 AM

Bioengineering MSC-EVs Advancing Regenerative Medicine through Cellular and Vesicular Therapies

Sujata Mohanty[1], Meenakshi Mendiratta[1]

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IntroductionMesenchymal Stem cell-derived small extracellular vesicles MSC sEVs are promising cellfree therapeutic in regenerative medicine However their efficacy faces several challenges such as nonspecific cargo packaging, loss in circulation and target specificity Current study employ dual strategies to modify surface and cargo of sEV to enhance their homing, target specificity and specific cargo enrichment for liver diseaseMethodsMSCs derived from bone marrow BM and Wharton’s jelly WJ were isolated following donor consent ICSCR14023, IECPG8707.03.2023 MSCs were cultured in serum-free media to isolate sEV via ultracentrifugation and characterized per MISEV2023 guideline sEVs were bioengineered by 1 surface modification: hepato-specific ligand via chemical conjugation; 2 cargo enrichment: hepatoprotective miRNAs via lipofection Provisional Patent applied Surface modification of bioengineered sEVs BioEn-sEVs was confirmed by lectin-induced aggregation and cargo enrichment validated by qPCR. Invitro studies assessed cellular uptake of BioEn-sEVs in Hepatic HuH7 and Non-

Hepatic Cell Human skin fibroblast IHF and immunomodulatory effect on Tcell proliferation, Treg induction and macrophage polarization PBMNC ROS reducing ability of BioEn-sEV was analyzed by MitoSOX in H2O2treated HuH7ResultsBioEn-sEVs retained characteristic features similar to naïve sEVs but had larger diameter 193nm compared to naïve sEVs 49nm BioEn-sEVs exhibited significantly enhanced uptake in HuH7 cells compared to IHF and suppressed CD8T cell proliferation, promoted Treg differentiation $p<0.0001$, and polarized macrophages towards the M2 phenotype $p<0.0001$ BioEn-sEVs significantly reduced oxidative stress compared to naïve sEVs $p<0.001$ WJderived BioEn-sEVs performed better than naïve counterpart, demonstrating potent hepato-regenerative potential through miRNA enrichment and surface modification displayed synergistic effect and enhanced their therapeutic potential for liver disease.

10:45 AM - 11:00 AM

Engineered Extracellular Vesicles for Therapeutic Applications

Natalia Higuita-Castro, Department of Biomedical Engineering & Department of Neurosurgery - The Ohio State University

Over the past two decades, nanoscale technologies have emerged as a promising tool to enable groundbreaking biomedical research and translational applications. We will discuss the use of nanotechnology-enabled strategies to develop novel nanocarriers based on engineered extracellular vesicles (EVs) to target cells/tissues. EVs are cell-derived carriers that share molecular cues with their donor cell and play a crucial role in cell signaling under healthy and pathological conditions. The implementation of engineered EVs as next-generation non-viral nanocarriers for gene and drug delivery helps to circumvent major practical and translational barriers inherent to other delivery methodologies, such as capsid size and redosing restrictions for viral vectors. Moreover, EVs possess an innate ability to penetrate biological barriers, have low immunogenicity, and higher stability in biofluids compared to synthetic carriers. Our data highlights the potential of engineered EVs to drive direct reprogramming processes of somatic cells towards induced endothelial or neuronal tissue, as well as their ability to reduce inflammation and facilitate tissue repair/regeneration.

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Featured Abstracts

11:10 AM - 12:30 PM

Evaluation of disparate methods to separate extracellular vesicles from multiple biofluids in the extracellular vesicle quality control (EVQC) study

Cláudio Pinheiro[1,2]: claudio.pinheiro@ugent.be

EVQC consortium members: Olivier De Wever[1,2] and An Hendrix[1,2]

[1]Laboratory of Experimental Cancer Research, Department of Human Structure and Repair, Ghent University, Ghent, Belgium. [2]Cancer Research Institute Ghent, Ghent, Belgium. # TRAIN-EV Marie Sklodowska-Curie Action-Innovative Training Network, train-ev.eu.

Introduction: EVs exert multiple biological functions which has spurred their development as disease biomarkers and drug delivery vehicles. Multiple methods have been developed to prepare EVs from biological samples exploiting different biophysical and biochemical characteristics such as size, density and surface antigens. However, no appropriate and quantifiable performance metrics are available which hampers their informed selection.Methods: In the EVQC study we developed an online guiding tool populated by results of a systematical comparison of 13 EV preparation methods for 3 biofluids, including cell culture supernatant, urine and blood plasma. We used a complementary array of characterization methods, including mass spectrometry-based proteomics, small and total RNA sequencing, high resolution flow cytometry, RT-qPCR, nanoparticle tracking analysis (NTA) and electron microscopy (EM), to assess EV preparations in terms of efficiency, specificity, repeatability, biomolecular landscapes and functionality at particle, protein, nucleic acid and structural levels.Results: We developed robust quality metrics to objectively assess the performance of EV preparation methods. We identified method-dependent efficiency, specificity, repeatability and biomolecular landscape (including biomolecular corona) across biological samples. We empirically demonstrate an invert correlation between efficiency and specificity alongside with high repeatability indexes (ALC>0.6). Proteomic landscape analysis revealed disparate pathway enrichment between healthy and cancer donors however not in all EV preparations. Small RNA seq analysis of biofluids derived from healthy and disease conditions allowed us to measure differences of up to 10000-fold miRNA concentrations.Conclusion: The results indicate that each method has its strengths and weaknesses. The identification of such idiosyncrasies will help to guide the informed selection of EV preparation methods for particular study goals.

Single Extracellular VEsicle Nanoscopy-Universal Protocol (SEVEN-UP): accessible imaging platform for quantitative characterization of single extracellular vesicles

Benjamin Purnell[1], Andras Saftics[1], Balint Beres[1,2], Nan Jiang[1], Ima Ghaeli[1], Carinna Lima[1], Brian Armstrong[3], Rupangi Vasavada[4], Tijana Jovanovic-Talisman[1]

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Extracellular vesicles (EVs) are membrane-encapsulated nanoparticles shed from all cells. They are tightly involved in critical cellular functions and have recently emerged as exciting therapeutic delivery vectors and biomarker sources. However, EVs are difficult to characterize because they are typically small and heterogeneous in size, origin, and molecular content. Recent advances in single EV methods have addressed some of these challenges by providing sensitive tools for assessing individual vesicles; one example is our recently developed Single Extracellular VEsicle Nanoscopy (SEVEN) approach. However, many tools for the assessment of individual EVs are not universally available to the general research community as they typically require specialized equipment. Here, we show how single EV

studies may be democratized via a new method that employs super-resolution radial fluctuations (SRRF) microscopy and new analysis algorithms. SRRF is compatible with a wide range of microscopes and does not require specialized fluorophores. We herein quantified individual EVs by combining affinity isolation of EVs with SRRF microscopy and new analysis algorithms supported by machine learning. We first optimized the workflow and validated SRRF data obtained on widefield and total internal reflection fluorescence microscopes by correlating these images with single-molecule localization microscopy images. We further demonstrate that our approach, which we call SEVEN-universal protocol (SEVEN-UP), can robustly assess the concentration, size, and content of recombinant and crude plasma EVs. Finally, we used the platform to assess EVs enriched in selected glycosylation residues. Altogether, we developed an economical, multiparametric, single EV characterization approach for the research community.

Scalable and reproducible EV isolation from patient samples using plate-based SEC and automation

Tal Gilboa[1,2], Dmitry Ter-Ovanesyan[1], Babila, Clarissa May[1], Shad Morton[1], Jenny Tam[1], David Kalish[1], George M Church$_{1,3}$, David R Walt[1,2,3]

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Extracellular vesicles (EVs) are released by all cells into biofluids and hold great potential as a new class of biomarkers. The utility of analyzing EVs in clinical samples has been hampered, however, by a lack of suitable EV isolation methods that are scalable to large numbers samples and yield EVs of high yield and purity. We previously developed Single Molecule Array (Simoa) digital ELISA assays for CD9, CD63, CD81 to measure EVs and Albumin and ApoB100 to measure non-EV contaminants. Using these assays, we found that SEC is superior to other methods, and were able to optimize SEC parameters such as resin, column volume, and fraction collection. With our SEC method in place, we set out to increase the throughput of SEC by engineering hardware that allows running multiple SEC columns in parallel. First, we built a semi-automated device that runs 8 columns in parallel. Next, to continue increasing the throughput further, we adapted SEC to run in 24-well plates and built hardware to integrate these 24-well SEC plates with liquid handling platforms for fully automated EV isolation. We've developed novel methods for reproducibly isolating EVs using highly optimized SEC from plasma and other biofluids that can be scaled to hundreds of samples, enabling the use of EVs in biomarker discovery and diagnostics.

Bulk analysis of extracellular vesicles by synchrotron-based Fourier Transform Infrared Spectroscopy

William Phillips[1], Eduard Willms[1], Jereme Spiers[2], Natasha Vassileff[2], Irumi Amarasinghe[1], Ebony Monson[1], Karla Helbig[1], Nicholas P. Reynolds[1], Lesley Cheng[1] and Andrew Hill[3]

[1]La Trobe University, Bundoora, Australia. [2]Australian National University, Canberra, Australia. [3]Victoria University, Melbourne, Australia

Analysis of extracellular vesicles (EVs) relies on detecting analytes within heterogeneous populations of EVs. This heterogeneity can arise from disease-induced changes, variations of sub-populations due to isolation methods, and the co-isolation of non-vesicular particles such as lipoproteins and lipid droplets.

Fourier Transform infrared spectroscopy (FTIR) is a bulk measurement technique that can detect composition changes in EVs and provide insight into macro changes in a total population of isolated EVs. In this study, synchrotron-based scatter FTIR with Baf2-coated slides was used to compare different populations of EVs. Murine neuroblastoma cell (N2a)-derived EVs were isolated using different techniques, including size exclusion chromatography, density gradient ultracentrifugation, ion exchange chromatography, and monolith-based CD81 affinity isolation. Analysis of FTIR spectra and principal component analysis (PCA) of integrated second derivative spectra revealed distinct differences between some isolation methods. It allowed for the differentiation of isolated EVs from lipid droplets. In addition, FTIR was applied to brain-isolated EVs from Alzheimer's disease (AD) patients to explore potential fingerprints associated with AD. These results demonstrate the heterogeneity and differences in EVs isolated by different methods and from cells under different treatments. These findings highlight the significant heterogeneity of EVs isolated by different methods and under varying conditions, underscoring the potential of FTIR as a powerful characterisation tool for EVs, especially as additional high-resolution techniques become available.

Novel fluorescence resonance energy transfer (FRET)-based assay to measure extracellular vesicle content delivery

Sarah Marquez[1], Zach Troyer[1], Martina Fabiano[1], Olesia Gololobova[1], Kenneth Witwer[1]

[1]Johns Hopkins University, Department of Molecular and Comparative Pathobiology

Introduction: Extracellular vesicles (EVs) are thought to have the ability to transport and deliver cargo between cells. However, for native EVs, data suggest a low efficiency of endosomal escape and content delivery. Therefore, engineering EVs with improved content delivery capacity to target cells is a growing focus of attention in therapeutics research. Assessing the effectiveness of such modifications is often hindered by the lack of methodology standardization and availability of reliable content delivery assays in the field. For this reason, we designed a novel EV-based variant of a 1998 viral fusion assay to address the need for a reliable EV-content delivery assay. Methods: EVs were isolated from Expi293 cells expressing Beta Lactamase (BlaM) fused to enhanced green fluorescent protein (EGFP) and a palmitoylation tag that secures this construct (PGB) to the EV inner membrane. Viral envelope proteins were co-expressed with the PGB construct to enhance EV content delivery. HEK293T target cells were loaded with CCF2, a FRET dye composed of 7-hydroxycoumarin and fluorescein joined by a beta-lactam ring which can be cleaved by BlaM. CCF2-loaded target cells were incubated with PGB-containing EVs. EV/cell membrane fusion allows BlaM exposure to cytosol, cleaving CCF2. Flow cytometry was used to measure the uncleaved vs cleaved dye signal intensities to obtain a measurement of EV content delivery. Results: VSV-G PGB EVs had a ~47-fold higher rate of content delivery to cells compared to PGB EVs without viral modification. Interestingly, PGB EVs only had a content delivery rate ~1.3-fold higher than the background of the assay, supporting the suggestion that native EVs rarely deliver their contents. Conclusions: The PGB assay represents an accurate and reliable alternative for the measurement of EV content delivery to target cells. It could be used to assess the effectiveness of therapeutic modifications designed to increase EV content delivery.

EVs derived from intravenous immunoglobulin (IVIg) therapy inhibit IFNg activation

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Departments of: [1]Pharmacology, Physiology, and Cancer Biology, [2]Otolaryngology – Head and Neck Surgery, and [3]Microbiology and Immunology, Thomas Jefferson University, Philadelphia, PA, USA; and [4]Department of Dermatology, University of Rochester, Rochester, NY, USA.

Intravenous or subcutaneous immunoglobulin (IVIg/SCIg) replacement therapy, derived from concentrated, pooled plasma of healthy donors, is used to boost the immune system in immunodeficiency states. IVIg is also highly effective in patients with autoimmune, infectious, and inflammatory diseases; however, with unresolved mechanism of action. Here, we suggest a novel mechanism of immunomodulation through extracellular vesicles (EVs). EVs were isolated from unprocessed normal human plasma (NHP), IVIg, and SCIg by size exclusion chromatography (SEC). Particle size and number were determined by NTA and ZetaView. IVIg/SCIg contained fewer EVs compared to NHP and the EVs were on average slightly larger, but within the range of exosomes. The protein corona (PC), an external layer composed of biological molecules, has emerged as critical for EV communication. Immune profiling of intact EV PC was performed by Luminex assay, which revealed that IVIg-derived EVs contained reduced levels of 31 pro-inflammatory cytokines compared to NHP (21 in SCIg), with 17 of these shared between IVIg and SCIg. Notably, RANTES, a potent inflammatory mediator, was dramatically reduced in IVIg/SCIg. Tangential flow filtration prior to SEC reduced immunoglobulin proteins but did not affect the PC cytokine content in the EVs. Interestingly, 9 cytokines were elevated in IVIg EVs (7 in SCIg), with 4 including IFNg being common between IVIg and SCIg EVs. The levels of IFNg were confirmed by flow cytometry and Western blotting. IVIg has been shown to block IFNg-mediated signaling in macrophages and T cells possibly through downregulation of the IFNGR2 receptor. Here, we demonstrate that incubation with IVIg EVs inhibited IFNg-induced STAT1 phosphorylation. In summary, the findings here suggest a potential anti-inflammatory mechanism of IVIg/SCIg that is mediated through EVs either by reducing pro-inflammatory cytokines associated with the PC or by inhibiting IFNg-mediated activation of JAK/STAT pathway.

Interoperable and Complementary Bead-Based Technologies for Extracellular Vesicle Subset Characterization

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Background: EVs are released from virtually all cells and carry diverse cargo related to their biogenesis and origin. The characteristic of EVs to carry surface markers from their parental cell enables these markers to be used to interrogate cell type-specific EVs. Among these methods, bead-based assays have excelled due to several features: 1) improving the EV signal detection by colocalizing signals from numerous EVs of interest; 2) customization of targeted marker/EV subsets; and 3) variety of downstream analyses possible. We aimed to develop bead-based methodologies for characterizing specific populations of extracellular vesicles (EVs) based upon their surface repertoires. To this end, we

aimed to emphasize assay interoperability, reproducibility through calibration and complementarity.Methods: We refined and compared three bead-based approaches: 1) a kit-based Multiplex Analysis (MPA; Miltenyi), 2) structured hydrogel nanoparticles called Nanovials (Partillion), and 3) magnetic streptavidin beads (Pierce), for the immunocapture of EVs from multiple sample sources and different downstream applications.Results: Through cross-calibrated flow cytometry, MPA showed highly sensitive and exceptionally reproducible data of 39 different EV markers simultaneously from heterogeneous samples, including from various biofluids. Alternatively, Nanovials were capable of analyzing 1-2 targeted markers on EVs at a time by calibrated flow cytometry and were entirely customizable with biotinylated capture antibodies of choice. Semi-quantitative analysis indicated >1e6 potential binding sites on Nanovials for targeted EVs. Finally, magnetic streptavidin bead-based pulldowns showed excellent results for downstream cargo-focused assays (PCR, RNASeq, and Western blots) of EV subsets.Conclusions: Our results highlight many of the benefits, pitfalls, and best practice usage of these complementary techniques, which are valuable additions to the EV research toolkit.

Establishing an Extracellular Vesicle Fusion Platform for Targeted Central Nervous System Disease Treatment

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In therapeutics development, extracellular vesicles (EVs) have been applied as engineerable nanotherapeutics. However, EVs are heterogeneous, especially across cell types, limiting efficacy. Additionally, single EV characterization tools have not been rigorously applied to EVs. This project tackles these gaps in the field by optimizing an EV-EV fusion platform to create and characterize hybrid EVs. As a proof-of-concept, hybrid EVs were developed to target central nervous system (CNS) diseases by optimizing for brain cell uptake, neuroprotection, and immunomodulation.MethodsPlacental-derived mesenchymal stem cells (PMSC) amd human astrocytes were cultured for EV isolation. EVs were isolated via ultrafiltration using Exodus, a new technology with improved yield and purity compared to ultracentrifugation. PMSC-EVs and astrocyte EVs (AEV) were fused via extrusion or sonication.EV composition was assessed through super resolution microscopy, ExoView, and quantitative proteomics. Functional properties of hEVs were measured via neuroprotection, cell uptake, and immunomodulation assays.ResultsA 1:1 ratio of PMSC-EVs and AEVs was optimal for fusion efficiency via super resolution microscopy. Sonication hEVs had higher fusion efficiencies than extrusion hEVs. Neither fusion method altered hEV surface protein expression as seen by ExoView; however, extrusion hEVs had less luminal protein cargo via proteomics analysis. hEVs had increased cellular uptake with increasing AEV ratio in a primary triculture model of neurons, astrocytes, and microglia. Conversely, hEVs had increased neuroprotective and immunosuppressive effects with increasing PMSC-EV ratio in a SY5Y neuroprotection model and activated glial model, respectively.We optimized a EV fusion platform to combine functions present in separate EV populations into a single hEV formulation. Using PMSC-EVs and AEVs, we demonstrated the ability to develop multifaceted EV-based nanotherapeutics for CNS diseases.

A low-cost method for gentle, effective and timely extracellular vesicle (GET EVs) isolation: accelerating development of RNA-based liquid biopsies

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Extracellular vesicles (EVs) are small fragments released by all types of cells, making them promising candidates for developing biomarkers for disease diagnosis and prognosis. Effectively isolating ultra-pure EVs for subsequent analyses is essential for the development of advanced EV-based liquid biopsies. Unfortunately, existing isolation methods including ultracentrifugation (UC) and various commercial kits, are lackluster due to damage to EVs, lengthy processing times, and low EV recovery rates. In response, we developed an "GET EV" method or kit which is based on a class of fluids called aqueous two-phase system (ATPS), which can selectively partition EVs to particular fluid phases based on the surface properties. This easy-to-practice method isolates EVs rapidly (within 20 min) from a diverse volume range of EV-containing biofluids (0.5 to 100 mL). Nanoscale flow cytometry (nFC) analysis revealed that GET EVs has greater EV enrichment capability (21.4X vs. 10.9X times fold enrichment) and higher EV recovery efficiency (97.6% vs. 69.3% recovery) than UC. Transmission electron microscopy revealed that EVs isolated by GET EVs are monodispersed with no aggregation as occurs in UC isolated EV samples. Commercial EV isolation kits were tested and were inferior to GET EVs in terms of EV recovery and EV-RNA recovery. EV subpopulations as determined by EV biomarkers (CD9, CD63, CD81, etc.) were confirmed by nFC. Small RNA sequencing of EVs isolated by GET EVs revealed highly representative miRNA, circRNA and snoRNA profiles compared to parental cells and EVs isolated by UC and commercial kits. Our study establishes the use of GET EV method/kit to efficiently, rapidly, and gently isolate EVs from various types of EV-containing biofluids (tissue culture media, human plasma, etc.). This is a low-cost innovation that will significantly enable the development of RNA-based liquid biopsies with significant diagnostic and prognostic implications.

Using the designer EV tookit (DEVKIT) as a framework to standardize EV engineering

Brian Dobosh[1], Milagros Parilla[1], Prashant Kumar[1], Ziya Jiwani[1], Emma Kingwell[1], Alexander Rojas[1], Rabindra Tirouvanziam[1]

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Massive effort has been applied by the ISEV Rigor and Standardization Subcommittee and the ISEV community at large to improve all facets of EV production. To enhance sharing of EV engineering molecular biology tools we have created the designer EV tookit (DEVKIT). The DEVKIT is a collection of common promoters, genes/open-reading-frames, and 5'- and 3'- untranslated regions (UTRs) mined from existing synthetic biology and EV literature cloned to be modular and swappable. All components are submitted to Addgene, a non-profit repository, to ease dissemination. It is our intention that by having the DEVKIT available, EV researchers will now be able to utilize a common framework to generate engineered EVs with desired protein (internal, transmembrane, or corona) and RNA (mRNA and others) with custom targeting abilities (transmembrane domains fused to single-chain variable fragments). To demonstrate the utility of the DEVKIT we set up three demonstrations using HEK293T cells: 1) Ranked loading of EGFP and BRET proteins conjugated to multiple pro-EV proteins: CD63, PTGFRN, Basp1aa1-30, Rab27, VSV-G and others into EVs; 2) Loading custom mRNA (EGFP or Cre) with highly stabilized 3'UTRs for long-expression using the RNA-binding protein L7Ae bound to the aforementioned pro-EV proteins; 3) Transfection of three modified HEK293T lines to produce EVs with different single-chain variable fragments fused to PTGFRN and different fluorescent proteins to enable tracking and delivery of EVs with various targeting properties. EVs were purified by 300 KDa tangential flow filtration (TFF). Structure, size, concentration, contents and contamination were evaluated by transmission electron microscopy, nanoparticle tracking analysis, western blotting, nanoflow cytometry, and qRT-PCR in concordance with MISEV2023. Delivery of cargo to primary human neutrophils, HEK293T, and H441 lung epithelial cells was assessed similarly.

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Oral Presentations

Saturday, November 23

9:15 AM - 9:30 AM

Engineering stem cell-derived extracellular vesicles for targeted treatment of central nervous system disorders

Aijun Wang, University of California Davis

Stem cell-derived extracellular vesicles (EVs) have remarkable therapeutic and regenerative potential. However, translational application of EVs has been limited for central nervous system (CNS) disorders due to their limited ability to cross blood brain barrier and rapid degradation and diffusion after systematic administration. We have developed a series of technologies to improve EVâ€™s CNS delivery efficiency and cell-type and extracellular matrix specific targeting and delivery. Our lab has generated extensive scientific evidence that human placental mesenchymal stem cells (PMSCs) and their derived EVs (PMSC-EVs) demonstrate efficient delivery of neuroprotective cargo to regions of CNS injury with significant improvement of neurological outcomes in various models, including neuroprotection assays in vitro, rodent models of multiple sclerosis, as well as in an ovine model of spina bifida. Based on these

encouraging results, we have initiated a first-in-human clinical trial to treat spina bifida in utero with PMSCs. We have demonstrated that PMSCs and their EVs contain neuroprotective and immunomodulatory cargo that can support neuroprotection, limit myelin damage, and inhibit neuroinflammation. Recently, we have established a near-term ovine model of neonatal hypoxic-ischemic encephalopathy. Preliminary studies in this large animal translational model confirmed safe and highly efficient delivery of PMSC-EVs to the brain following intranasal administration, circumventing the significant bottleneck of crossing the blood-brain barrier, and demonstrated disease-modifying activities. To tailor EV biodistribution in vivo and further enhance the delivery efficiency of EVs to the CNS, we modified the surface of EVs with cell-type and extracellular matrix specific binding ligands (e.g. peptide or aptamer). We established single EV characterization technologies to measure EV surface conjugation efficiency and optimize the modification process for improved targeting.

9:30 AM - 9:45 AM

Use of programmable milk extracellular vesicles to deliver therapeutics

Janos Zempleni[1], Alice Ngu[1], Qamar Taban[1]

[1]University of Nebraska-Lincoln

Milk extracellular vesicles (MEVs) possess qualities conducive to delivering therapeutics to pathological tissues. As is the case for most nanoparticles, the use of MEVs in nanomedicine is limited by their rapid elimination by macrophages and imperfect homing to target tissues.We have developed “programmable” MEVs (PMEs) in which non-canonical amino acids (ncAAs) are substituted for natural amino acids in CD81 loops; the protein is engineered to express a poly-his tag for purifying MEVs through a Ni2+ resin. After MEV purification, the ncAAs serve as docking sites for synthetic Don’t-eat-me peptides and homing peptides using click chemistry protocols. We have tested and optimized the technology in mammary epithelial cells which secrete extracellular vesicles with properties nearly identical to MEVs.Lentiviral vectors were used to engineer mammary cells that secrete PMEs. When PMEs were decorated with Don’t eat me peptides, CD24 or CD47, the uptake of MEVs by primary macrophages was reduced by ~50% ex vivo, and peak serum concentrations in mice were up to 12.3-fold higher compared to unmodified PMEs following tail vein injection ($p< 0.05$; $n = 5$). The decreased PME elimination was associated with a substantial increase in the accumulation of PME in the brain. When MEVs were loaded with a plasmid encoding near-infrared fluorescent protein (iRFP) and delivered by nasal spray, iRFP was expressed in the brain. When PMEs were decorated with glioma homing peptides, CooP or apoE, the particles accumulated exclusively in pathological cells in a xenograft mouse model of human glioma. When PMEs were loaded with a plasmid encoding Syngap1 and delivered to Syngap1 mutant mice by nasal spray, Syngap1 mRNA and protein expression increased by 12-fold and 17-fold compared to controls. Programmable PMEs have the potential of becoming a game changer in nanomedicine by enabling the treatment of virtually all rare and common diseases.Support: NIH, NIFA, USDA, SynGAP Research Fund.

10:30 AM - 10:45 AM

Rationally designed bioreactor for enhanced MSC EV bioactivity and production

Steven M. Jay, University of Maryland Department of Bioengineering

MSC EVs are implicated as therapeutics for many applications. However, several limiting factors to translation exist, including challenges retaining potency with scale-up production. While many genetic and biochemical priming approaches have been employed to boost MSC EV efficacy, MSCs are a mechanoresponsive cell type, and mechanobiological stimuli could be more straightforwardly and cost-effectively incorporated into an EV biomanufacturing process. While much work has been done in this area related to substrate stiffness and flow/shear effects, we have demonstrated the potential of a different mechanobiological parameter, physical confinement. MSCs under confinement are forced into an elongated morphology, which correlates with a therapeutic phenotype for these cells. In microfabricated devices, we have shown that EVs from confined MSCs have increased therapeutic bioactivity (improved stimulation of angiogenesis, increased anti-inflammatory effects). We have now created a bioreactor wherein MSCs can be grown under confinement and also subjected to media flow. Our initial results show that this novel bioreactor can be used to generate large quantities of therapeutically enhanced MSC EVs compared to standard culture techniques. Here, we will discuss the design and development of this rationally-designed bioreactor as well as report the latest data from the EVs it produces.

10:45 AM - 11:00 AM

Developing tools and animal models for non-invasive detection of exhaled lung tumor extracellular vesicle biomarkers

Megan I. Mitchell[1,2], Iddo Ben-Dov[3], Christina Liu[1], Rachel B. Hazan[4], Johannes Zakrzewski[1,2], Jeffrey Luo[5], Russell Seth Martins[5], Faiz Bhora[5], Thomas Bauer[6], Kathryn Donnelly[1], Matan Peleg[1], Junfeng Ma[7] and Olivier Loudig[1,2]

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The field of breath biopsy shows promise for the detection of lung tumors. The analysis of both volatile organic compounds (VOCs) and non-volatile organic compounds (non-VOCs) present in exhaled breath has been associated with the detection of metabolic and biologic changes in the lung. While the collection and analysis of exhaled VOCs must be performed in sync using electronic equipment that provides quantitative measure on its chemical composition, the collection of non-VOCs can be done simply by condensation of exhaled breath, which provides biofluid that can be stored and later undergo molecular analyses. Human exhaled breath condensates (EBC) are collected non-invasively and very rapidly by condensation of exhaled vapors that are produced during normal tidal respiration. EBC

studies have revealed that it contains microRNAs (miRNAs), which isolation and profiling have been shown to reflect the condition of the lung. However, recent investigations, including ours, have revealed that EBC also contains exhaled extracellular vesicles (EVs), which cellular origin can be traced to the bronchoalveolar unit, and that thus carry diagnostic potential for the detection of lung diseases including cancer. Although the isolation and analysis of human EBC and exhaled EVs have potential for non-invasive detection of lung cancer, there are currently no EBC biobanks. Considering that lung cancer is an indolent disease that is difficult to detect, the collection of relevant EBC samples from patients with different types of lung tumors will take time. Thus, in order to evaluate the potential of exhaled microRNA biomarkers for non-invasive detection of lung cancer, we conducted the collection and analysis of EBC from an orthotopic lung tumor mouse model during disease development. Our analyses reveal that EBC can be reproducibly collected from mice, analyzed, and in turn identify miRNA signatures that allow early and non-invasive detection of aggressively developing lung tumors.

11:00 AM - 11:15 AM

Scalable trapping and label-free characterization of single extracellular vesicles in solution

Ikjun Hong[1], Theodore Anyika[1], Maxwell Ugwu[1], Abayomi Opadele[1], Justus Ndukaife[1]

[1]Department of Electrical and Computer Engineering, Center for Extracellular Vesicles, Vanderbilt University

Extracellular vesicles (EVs) and non-vesicular extracellular nanoparticles (NVEPs) are now recognized as vital mediators of cell-to-cell communication. They transport essential molecules like mRNA, miRNA, proteins, and lipids, facilitating both local and long-distance signaling. However, their diverse origins and sizes pose a challenge for understanding their roles in health and disease. Traditional optical tweezers face limitations for trapping single EVs due to the small size of EVs and NVEPs and the diffraction limit of light. In this discussion, I will introduce new innovative optical nanotweezers that can rapidly trap nanoscale EVs and supermeres in parallel within seconds. Furthermore, I will discuss the nanotweezer-assisted label-free characterization of the trapped EVs and extracellular nanoparticles while they are held at their respective trapping sites. These novel non-invasive light-based tools with multifunctional capabilities are expected to open new horizons by enabling to address fundamental questions in EV biology and drive translational applications.

2:15 PM - 2:30 PM

Single Biomarker Profiling for Medical Diagnostics

Jina Ko, University of Pennsylania

Due to an inherent biological heterogeneity across individuals and within a disease, it is extremely challenging to identify robust biomarkers that can accurately represent molecular status of the body for disease diagnostics. To solve this intractable problem, we have developed microfluidic platforms and molecular tools that enable high throughput, multiplexed profiling of biomarkers (e.g. cells, extracellular vesicles; EV). We achieved high throughput profiling by combining sequencing with parallelization of

microchip technologies and droplet microfluidics. We overcame the variability of any individual biomarker between individual patients, by developing tools that can measure multiple markers and we applied machine learning to identify signatures that persist across this variability. To resolve cell and EV heterogeneity, we have recently developed an ultra-fast cycling method for single cell analysis and an ultra-high sensitive microfluidics that can achieve single particle detection sensitivity, enabling individual EV measurements.

2:30 PM - 2:45 PM

Membrane Scaffolds for Exosome Imaging and Cargo Loading in Living Human Cells

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Background: Exosomes, a new generation of nanoscale vesicles, have garnered significant interest for drug delivery and therapy due to their intrinsic biocompatibility, high tissue-penetrating ability, and de novo programmability for tissue targeting and cargo loading. Membrane scaffolds play a crucial role in transforming exosomes for targeted drug delivery and therapy. However, despite the availability of several membrane scaffolds, a comprehensive evaluation of their performance in the genetic programming of exosomes has been lacking.Methods: We designed and constructed fusion reporters incorporating various membrane scaffolds to assess their performance in exosome imaging and cargo loading in cultured human cells.Results: Our findings reveal that all three transmembrane scaffolds tested—CD63, LAMP-2B, and vesicular stomatitis viral envelope glycoprotein (VSVG)—exhibited high specificity and efficacy in exosome imaging and cargo loading, confirming their usefulness in exosome engineering. In contrast, these four membrane-associated scaffolds—acylation signal peptide (ACY), farnesylation signal peptide (FAR), glycosylphosphatidylinositol (GPI) signal peptide of DAF, and the C1C2 domain of lactadherin—showed varying levels of specificity and efficacy. Notably, the smallest FAR (12 amino acids) demonstrated the highest specificity and efficiency in both transient and stable cell models. Additionally, the FAR scaffold successfully loaded three functional proteins (Gaussia luciferase, green fluorescent protein and thymidine kinase enzyme from human herpesvirus) into the lumen of exosomes, showcasing its robustness in exosome imaging and therapeutic cargo loading.Conclusion: Our study comprehensively examines the specificity and utility of available membrane scaffolds in exosome imaging and cargo loading in human cells. The insights gained will aid in selecting the appropriate scaffolds for different applications in exosome research and therapy.

2:45 PM - 3:00 PM

Direct ultrasensitive analysis of extracellular vesicles in plasma samples using membrane sensing peptides as pan-affinity probes

Alessandro Gori[1], Paola Gagni[1], Roberto Frigerio[1,2], Jacopo Burrello[3], Stefano Panella[3], Greta Bergamaschi[1], Giulia Lodigiani[1], Andrea Zendrini[2], Miriam Romano[2], Annalisa Radeghieri[2], Lucio Barile[3] and Marina Cretich[1]

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Extracellular vesicles hold immense potential for diagnostic applications. However, challenges in isolating EVs from complex biological specimens hinder their widespread use. Additionally, the detection of low-abundant EV populations demands for highly sensitive and high-throughput technologies. In this context, integrated isolation-and-analysis workflows based on immunoaffinity represent the go-to strategy. Nevertheless, the high heterogeneity of EVs and the use of antibody-based tools inherently drive to EV subpopulations pre-selection, raising concerns about the reliability of downstream biomarker discovery analysis. This issue extends to the increasing field of engineered EV-mimetics and bio-nanoparticles, where conventional immune-affinity methods may lack applicability. Addressing these challenges, we introduce a streamlined process integrating the use of Membrane Sensing Peptides (MSP) for EV capture and phenotyping through Single Molecule Array (SiMoA) technology. MSP work as "pan-vesicular" affinity ligands for both EVs and EV-analogues, binding the vesicle membranes agnostically and irrespective of the relative abundance of protein surface markers. We showcase the application of MSP-SiMoA in the integrated analyses of circulating EVs in blood derivatives (serum and plasma), eliminating the need for prior EV isolation. Demonstrating the possible clinical translation of MSP technology, we directly detect an EV-associated epitope signature in serum and plasma samples, demonstrating its potential for distinguishing patients with myocardial infarction versus stable angina. At last, notably, MSP exhibits a unique capability to enable the analyses of tetraspanin-lacking Red Blood Cell derived EVs (RBC-EVs). Taken together, these applications highlight the potential of MSP in advancing EV analyses for clinical diagnostics and beyond.

3:00 PM - 3:15 PM

Synergistic Effect of Combined EV Isolation Technologies: Charge-Based Filtration and Tangential Flow Filtration with Lipoprotein-Specific Adsorption Filter

Yongwoo Kim[1], KanfMin Lee[1], SoYoung Jeon[1], Sehyun Shin[1]

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This study introduces an innovative hybrid EV extraction technology by combining the charge-based exosome separation technology, ExoFilter, with lipoprotein-specific adsorption filter technology and integrating it with Tangential Flow Filtration (TFF), a technology that excludes nanoparticles below a certain size. ExoFilter, an ion-exchange based technology, was recently developed to process negatively charged EVs from small sample volumes to liter-scale samples. TFF, widely used for its unique ability to concentrate samples by excluding nanoparticles below a specific size, when combined with charge-based ExoFilter technology, achieved unexpectedly high recovery rates and high purity through iterative flow processes. Additionally, by incorporating a filter process with an Apop-B-specific aptamer, a surface

marker of lipoproteins, the previously obtained high-purity extracts were further refined to ultra-high purity without reducing recovery rates. This series of integrated processes retained the individual technical advantages, resulting in remarkable purity improvement and a synergistic effect that more than doubled the existing recovery rates. This represents a significant technological advancement in the extraction technology by efficiently removing lipoproteins, which are difficult to eliminate with conventional methods. The combined technology of each filter, scalable from small blood samples to hundreds of liters of culture medium, presents a new method for efficiently obtaining high-quality exosomes for various biological and medical applications.

Poster Sessions

Thursday, November 21 & Friday, November 22

5:00 PM – 7:00 PM

First Name	Last Name	Abstract Title	Day	Poster Number
Aslan	Dehghani	The Importance of Established and Optimized Analytical Capabilities to Develop End to End and Scalable Therapeutic Extracellular Vesicles Processing	Day 2	4
Boyang	Su	Precise isolation of specific EV sub-populations with the Kairos cLDEP sorter	Day 1	2
Erez	Eitan	Methods to measure plasma neuron-derived extracellular vesicle enrichment	Day 1	3
Huiqiao	Pan	The Role of Bacterial Membrane Vesicles in Soybean- Bradyrhizobium symbiosis	Day 1	4
Lin	Wang	Eneabling breakthroughs with EzerSonic - sEV Isolation instrument and service	Day 1	5
Martin	Schlumpberger	Evaluation of a Novel Magnetic Bead-Based Method for Isolation of Intact EVs or EV-RNA	Day 1	6
Pasquale	D'Acunzo	Technics to study mitovesicle molecular composition and cell-free functionality	Day 1	7
Patrícia	Gomes	Isolation of spontaneously-released brain extracellular vesicles and its implications for brain pathology	Day 1	8
Vinh	Lam	SmartSEC-DeLipo: A New Standard of Pure EV Isolation for Lipoprotein Containing Samples	Day 1	9
IMANE	BAICHE	Production and use of EVs depleted human platelet lysate to improve large, clinical grade compatible, production of therapeutic human cell-derived EVs	Day 1	10
Michael	LeClaire	A Scalable Production Method for Exosomes	Day 1	11
Nick	Pirolli	Genetically-programmed high-yield biomanufacturing of probiotic bacterial extracellular vesicles for IBD treatment	Day 1	12
Robert	Kirian	Biocalorimetry to predict EV production and improve biomanufacturing	Day 1	13
Stephen	Lenzini	Scalable GMP-compatible Process Solution for MSC-EV Purification with 10X Yield Improvements	Day 1	14
Wei	Fu	Scalable Production of Ovarian Cancer Cell-Specific Extracellular Vesicles Using Bioreactors	Day 1	15
Aiden	Jurcenko	High-resolution characterization of HIV and extracellular vesicles: combining smFISH and dSTORM	Day 1	16
Nan	Jiang	Multiparametric Profiling of HER2-enriched Extracellular Vesicles in Breast Cancer Using Single Extracellular VEsicle Nanoscopy	Day 1	17
Sebastian	Molnar	Identification and characterization of small HIV-1 particles released from a chronically infected T-cell line: size, content, infectivity, and phenotype	Day 1	18
Serina	Erxleben	Enhancing Fluorescence Stability in NTA: New Approaches for Analyzing Extracellular Vesicles	Day 1	19

First Name	Last Name	Abstract Title	Day	Poster Number
Shima	Nikanik DeHorta	Correlation Between Absolute Scatter Intensity and Size in NTA Measurements	Day 1	20
Tarini	Basireddy	A tale of two proteins: ApoE and Ago2 in the extracellular vesicle protein corona	Day 1	21
Zach	Troyer	SPIRFISH: A new single-particle analysis method for simultaneous protein and specific RNA detection	Day 1	22
Sebastian	Aguayo	Collagen glycation modulates bacterial extracellular vesicle production by caries-relevant oral biofilms	Day 1	23
Alexandre	Kitching	Automated High-Resolution TEM Analysis of Extracellular Vesicles (EVs) Using Object Segmentation and Classification with NMTX-a1	Day 1	24
Bryant	Nelson	Testing the Analytical Robustness of Microfluidic Resistive Pulse Sensing for Extracellular Vesicle (EV) Particle Number Concentration (PNC) Measurements	Day 1	25
Clayton	Deighan	Quantification of EV surface proteins with fluorescent labelling and single EV detection	Day 1	26
Evgenia	Kim	Label-free Characterization of Biological Nanoparticles	Day 1	27
Evgenia	Kim	Label-free Technology to Assess Protein Loadings on the Surface of Biological Sample	Day 1	28
John	Nolan	Single vesicle flow cytometry with calibrated light scatter resolves EV structural variants	Day 1	29
Martina	Fabiano	Novel Characterization of Phosphatidylserine-Positive Plasma Extracellular vesicles	Day 1	30
Mona	Shahsavari	Developing a practical protocol for determining the lower limit of detection in flow cytometry: Validation using novel beads with a flat size distribution	Day 1	31
Alicja	Głuszko	Lipidomic and transcriptomic approaches revealed glycerophospholipids as a signature of hypoxia-derived small extracellular vesicles (sEVs) in head and neck squamous cell carcinoma (HNSCC)	Day 1	32
Marcell	Palmai	Synthesis and application of silica hollow spheres for flow cytometry analysis of extracellular vesicles	Day 1	33
Mary Lou	Bailey (Midgett)	3D morphological characterization of extracellular vesicles	Day 1	34
NADIM	TAWIL	The Center for Applied Nanomedicine (CAN) - Integration of Analytical Technologies for Advanced Extracellular Vesicle and Particle Research	Day 1	35
Ramon	Castellanos-Sanchez	Advancing and Functional Characterization of Microvascular Endothelial Cell-Derived Extracellular Vesicles.	Day 1	36
Rodrigo	Orso	The role of extracellular vesicle dynamics in homeostasis: A cross-species approach	Day 1	37
Stephen	Lenzini	Analytical comparison of MSC-EV quality attributes across tissue sources and production platforms	Day 1	40
Antonio	Giuseppucci	Exploring the impact of cigarette smoke extract exposure on extracellular vesicles: a comparative analysis across CNS cells	Day 1	41
Ben	Josey	Interferometric Light Microscopy for for a Fast Characterization of Extracellular Vesicles	Day 1	42

First Name	Last Name	Abstract Title	Day	Poster Number
Eisuke	Dohi	Temporal variation in miRNA levels in mouse blood plasma	Day 1	43
Elena	Khomyakova	Introducing innovative technique and diagnostic platform for early detection of ovarian cancer through profiling of protein markers on urinary EVs	Day 1	44
Elena	Khomyakova	Bead-based flow cytometry: multiplex versus single marker analysis. Advantages and limitations	Day 1	45
Elise	Hickman	Investigating the Effects of Wildfire Smoke on Extracellular Vesicle Release and Cargo as Key Mediators of Wildfire Smoke Toxicity Using an Equine In Vivo Approach	Day 1	46
Jessie	Arce	Integrating the NanoFlow Repository and the exRNA Atlas facilitates FAIR sharing and integrative data analysis about exRNA and their carriers	Day 1	47
Madeline	Cramer	Elevating MSC-EV Analysis: Development and Qualification of a CD73 Bioactivity Assay	Day 1	48
Noa	Avni	Advancing Exosome-Based Therapies: Overcoming Key Manufacturing Challenges for Scalable Production and Process Robustness	Day 1	71
Ji-Su	Kim	Pomegranate-derived exosome-like nanovesicles containing ellagic acid reduce gut leakage and liver injury in MASLD	Day 1	72
Jae-Hee	Kwon	Human breast milk-derived extracellular vesicles promote the differentiation and mineralization of osteoblast MC3T3-E1 cells	Day 1	73
Ji-Su	Kim	Ginseng-derived exosomes-like nanovesicles reduce hepatic fibrosis	Day 1	74
Ahmed	Abdelgawad	Circular RNAs: the rising star and most dominant RNA cargo in extracellular vesicles	Day 2	49
Daniel	Humphrys	Computational protein design enables an engineerable EV platform technology	Day 2	50
Harrison	Rudd	Human endogenous retrovirus group K (HERV-K) HML-2 is an important modulator of extracellular vesicle (EV) dynamics in hepatocellular carcinoma cells	Day 2	51
Kerstin	Creutzberg	The role of EVs in the intergenerational transmission of paternal experiences	Day 2	52
Nada	Abumrad	Blood Fatty acids cross capillaries and reach tissue cells in small extracellular vesicles.	Day 2	53
Prashant	Kumar	Methodological Advancements in Extracellular Vesicle Trafficking: Optimization of Dye Staining and Cellular Uptake	Day 2	54
Sumeet	Poudel	Role of Gene Copy Numbers in Extracellular Vesicle Cargo	Day 2	55
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