BSEVs
BALTIC SOCIETY OF EXTRACELLULAR VESICLES

COMBIVET

PROCEEDINGS

Combivet & BSEV Joint Conference on Extracellular Vesicles

30th of September to 1st of October, 2022

Tartu, Estonia

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PROCEEDINGS OF FIRST COMBIVET AND BALTIC SOCIETY OF EXTRACELLULR VESICELS JOINT CONFERENCE 2022

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About COMBIVET & BALTIC SOCIETY OF EXTRACELLULAR VESCILES JOINT CONFERENCE 2022

The Combivet and Baltic Society of Extracellular Vesicles (BSEV) Joint Conference is organized by the COMBIVET ERA Chair of Comparative Medicine in the Institute of Veterinary Medicine and Animal Sciences of the Estonian University of Life Sciences and Baltic scoiety of Extracellular Vesicles. The program is supported by the European Union (Horizon 2020 research and innovation programme under grant agreement No 857418).

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WELCOME

I am extremely happy to welcome you to the first COMBIVET and BSEV joint conference 2022, organized by COMBIVET ERA Chair of Comparative Medicine in the Institute of Veterinary Medicine and Animal Sciences of the Estonian University of Life Sciences and Baltic Society of Extracellular Vesicles. I am also proud to say that this would be the 6th conference on Extracellular Vesciles (EVs) organized by the Fazeli Lab. Due to pandemic and its consequences, we hosted our last conference as a hybrid event, therefore on behalf of the organizing committe, I am delighted to welcome you all to a full physical meeting this year.

In recent years, EVs have gained a considerable interest across different scientific communities, particularly those working with cancer, infectious and inflammatory diseases. In addition, EVs have emerged as important mediators of cell-cell interactions, making them very attractive targets for theranostics. In the past EVs were a hot topic in just Human Medicine, now investigators in Veterinary Medicine and even those working with plants and microorganisms are becoming interested in working on this amazing new and attractive field of science.

Under the COMBIVET ERA Chair of Comparative Medicine in the Institute of Veterinary Medicine and Animal Sciences of the Estonian University of Life Sciences and previously, under the ERA chair of Translational Genomics program of the University of Tartu, we have formed a nucleus of collaboration between different researchers interested in studying EVs, characteristizing, and their possible role in a range of human and animal diseases and conditions including infertility and other reproductive disorders, osteoarthritis, and psoriasis. Subsequently, we have gained

substantial experience in different aspects of the purification and characterization of EVs. Most importantly, we have managed to publish our EV research studies in many reputed journals, created excellent collaborations and apply our knowledge about EVs to many new fields of life science and medicine. Recently with extablishment of Baltic Society of Extracellular Vesicles (BSEV), we started collaborating more with scientist and professionals working in in the Baltic region. Therefore, we believe that by organizing this conference, we could create a platform for sharing and intergrating multidischiplinary scientific knowledge, hence creating more opportunities for networking for collaboration and career development.

I would like to thank all the speakers, participants and organizing committee of the conference for your share of contribution to the success of the conference. I sincerely hope that the conference will provide a solid platform for all to engage in meaningful scientific debates and discussions. It is my sincere wish that Combivet and BSEV joint conference 2022 will be a resounding success.

Alireza Fazeli
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1.0. DAY 1: 31st of September 2022

DAY 1 Featured Speakers (FS)

FS 1.1 | Alireza Fazeli

ERA chair of Comparative Medicine, Estonian University of Life Sciences, Tartu, Estonia,
Professor of Clinical Genomics and Personalized Medicine, Faculty of Medicine, Tartu University, Tartu, Estonia,
Professor in Academic Unit of Reproductive and Developmental Medicine, Department of Oncology and Metabolism, The Medical School, University of Sheffield, Sheffield, UK

Biography

Alireza Fazeli has over 20 years of experience of conducting multidisciplinary research in world-class universities and research institutions around the globe. During this time, he has developed vast experience using different "OMICS" technologies such as genomics and proteomics in translational research in the field of immune, reproductive and developmental medicine. In particular he has sought to investigate the Periconception Environment and its effect on the offspring epigenetic profile, maternal interactions with gametes and embryos as well as understanding the mechanisms involved in the mediation of the innate immune system in the female reproductive tract in health and disease. He is also currently serving as the president of Baltic Socieity for Extracellular Vesicles which was founded with the aim of fostering Extracellular Vesicles (EV) research activities in Baltic Countries.

FS 1.2 | Augustas Pivoriūnas

Deputy Director for Science and Head of the Department of Stem Cell Biology, Centre for Innovative Medicine, Lithuania

Biography

Augustas Pivoriūnas graduated from Vilnius University Medical Faculty in 1998 (M.D.) and received PhD (2004) in Biochemistry from the Institute of Biochemistry in Vilnius. As a holder of Marie Curie fellowship (Contract Nr: HPMT-GH-00-00130-04) he spent 2 years (2002-2004) at the Department of Medical Microbiology, Linköping University, Sweden. He joined the Department of Experimental Medicine at the Institute of Experimental and Clinical Medicine in Vilnius as research scientist in 2005. From 2010 to 2019 he served as a senior research scientist at the State Research Institute Centre for Innovative Medicine (SRICIM) in Vilnius and from 2012 he was appointed as a Head of the Department of Stem Cell Biology. From 2014 to 2020 he served as a Deputy director for Scientific Affairs at the SRICIM and from 2019 holds position of Chief research fellow. From 2014 Augustas Pivoriūnas is the President of the Lithuanian Association of Stem Cell Researchers. Augustas Pivoriūnas is a co-founder and shareholder of the joint stock company Exosomica. Augustas Pivoriūnas leads an active group of scientists and PhD students and his research interests focus primarily on extracellular vesicles (EVs) derived from different types of adult stem cells and their applications in basic research and cell-based therapies.

FS 1.3 | Mandy Perffers

Research Lead, Department of Musculoskeletal Science and Ageing, Institute of Life Course and Medical Sciences, University of Liverpool, United Kingdom

Biography

Mandy Perffers obtained a degree in Animal Science at the University of Leeds, and then undertook her veterinary degree at The Royal Veterinary College, University of London and qualified as a veterinarian in 1995. Following an internship in reproduction at Glasgow University she then spent 11 years in industry and private practice before undertaking a PhD supported by the Wellcome Trust on 'Proteomic and transcriptomic signatures of cartilage ageing and disease'. Her fellowship continued by studying 'A Systems Biology Approach to Musculoskeletal Ageing'. She then obtained a Wellcome Trust Clinical Intermediate Fellow studying the role of small nucleolar RNAs in cartilage ageing and disease as well as the potential use of extracellular vesicles to treat equine osteoarthritis. Her research group at Liverpool is a mix of vets, scientists at master, PhD and postdoctoral level studying the role of epigenetics in musculoskeletal diseases in man, dogs and horses. They are additionally interested in the role of extracellular vesicles in osteoarthritis as biomarkers and potential treatments. Much of her research has started with 'omic' discovery experiments to identify molecules for mechanistic studies or biomarkers. They utilise many forms of 'omics' technologies in our research including mass spectrometry proteomics (label-free quantification, absolute quantification and mass spectrometry imaging), nuclear magnetic resonance metabolomics, microarray, RNASeq, small RNASeq, QuantSeqReverse, ribosomal profiling and DNA methylation arrays. She is currently section editor for omics for the Equine Veterinary Journal.

FS 1.4 | Marca Wauben

Professor of Intercellular Communication, Faculty of Veterinary Medicine, University of Utrecht, Netherlands

Biography

Marca Wauben is a pioneer in EV-mediated communication and an internationally recognized expert in EV-biology, isolation and characterization. Wauben has investigated EV-mediated communication in different systems and models, evaluated possible clinical applications of EVs and developed technology for EV characterization and isolation. The Wauben EV Lab unveiled novel aspects of EV-mediated communication involved in immune regulation and on the regulation of EV release, EV cargo (proteins, RNAs and lipids) and targeting. Within the STW-UU-Nutricia partnership project (Wauben PI), an isolation method for nano-sized EVs from human milk was developed and proteomic analysis of EVs led to the identification of a 'novel functional milk proteome', while with the ZonMW Enabling Technology grant the RNA content of milk-derived EVs was analysed. The Wauben lab discovered that milk EVs have immune modulatory capacities and can increase epithelial barrier formation and they pioneered in single EV-based flow cytometric analysis. Besides investigating fundamental cell biological/immunological aspects, they search for EV-based biomarkers and therapeutic applications of EVs. Wauben is actively involved in many national and global initiatives to build strong scientific EV-biology communities.

FS 1.5 | Reet Kurg

Professor of Molecular Biomedicine, Institute of Technology, Faculty of Science and Technology, University of Tartu, Estonia

Biography

Reet Kurg graduated in 1988 as bio-organic chemist and obtained her PhD in 2000 in molecular biology at the University of Tartu, Estonia. She initially worked on the molecular biology of papillomavirus replication before moving on to cancer testis antigens. Her current research is dedicated for understanding of the biological functions and underlying regulatory mechanism of MAGEA proteins expression in cancer cells. She is also active in the field of extracellular vesicles with the aim of finding biomarkers for cancer diagnostics and generating EVs with desired properties. From 2018 she is a Director of the Institute of Technology, University of Tartu and from 2021 Professor of Molecular Biomedicine in the same institute.

FS 1.6 | Aiste Jekabsone

Senior Scientist, Preclinical Research Laboratory for Medicinal Products), Cardiology Institute, Lithuanian University of Health Sciences, Lithuania

Biography

Aiste Jekabsone is a senior scientist in the Laboratory of Preclinical Drug Investigation of the Institute of Cardiology, Lithuanian University of Health Sciences (LSMU, Kaunas, Lithuania). She has MSc in Molecular Biology and Biotechnology from Vytautas Magnus University and a PhD in Biomedical science from the Lithuanian University of Health Sciences (2002), where she investigated cell death pathways in ischemic myocardium. As a Postdoc fellow at the University of Cambridge (UK), Biochemistry department, she worked on nitric oxide signalling pathways in stroke models and investigated the role of microglial cells in Alzheimer's disease. She currently focuses on designing extracellular vesicle-based technologies for drug delivery and regeneration and organotypic *in vitro* models for drug efficacy – toxicity screening.

FS 1.7 | Qurat Ul Ain Reshi

Junior Research Fellow, Institute of Veterinary Medicine and Animal Sciences, Estonian University of Life Sciences, Tartu, Estonia

Biography

Qurat Ul Ain Reshi pursued her B.Sc in Zoology & Industrial Chemistry and M.Sc in Clinical biochemistry from University of Kashmir, India. Later she did her Master´s dissertation in Special Centre of Molecular Medicine (SCMM), Jawarharlal Nehru University, New Delhi, India where she worked on DNA replication machinery of Plasmodium falciparum. In 2018, she joined the Department of Pathophysiology, University of Tartu and started her PhD studies under the supervision of Prof. Alireza Fazeli. In Tartu, she is working in a developmental biology lab where she aims to understand the role of extracellular vesicles in interactions between spermatozoa and oviductal epithelial cells in cattle.

FS 1.8| Sergei Kopanchuk

Research Fellow, Institute of Chemistry, University of Tartu, Tartu, Estonia

Biography

Over the past 20 years at the Universities of Tartu and Uppsala, Sergei Kopanchuk has gained expertise in studying the molecular mechanisms of the functioning of various G protein-coupled receptors, focusing on the dynamics of system behavior. He is involved in the development of various types of assays for drug screening and biosensors. In the last decade, he has built and configured microscopes with different fluorescence imaging modes (confocal, wide-field and TIRF/HILO) for various measurement techniques such as FLIM in time and frequency domain, TGL, FCS and single molecule/particle detection/tracking. In addition, he has enhanced these optical setups with super-resolution options, STED and localization microscopy.

FS 1.9 | Aija Linē

Associate Professor, Faculty of Biology, University of Latvia, Riga, Latvia
Group Leader, Latvian Biomdical Center, Riga, Latvia

Biography

Prof. Aija Linē is the head of Cancer Biomarker group, the scientific director of Latvian Biomedical Research and Study centre and professor at University of Latvia in molecular genetics and immunology. She obtained her PhD at the University of Latvia in 2002 on the identification and characterization of tumor antigens recognised by B cells. During her PhD and post-doctoral studies she undertook several training periods at Nottingham Trent University to study the molecular alterations underlying their immunogenicity. In 2004 she established her group focusing on the discovery of circulating cancer biomarkers. Currently, her research interests are focused on the extracellular vesicles as a source of cancer biomarkers for liquid biopsies and therapeutic tools for the treatment of cancer. She has published 55 peer-reviewed article cited >10700 times, her H-index is 26 and she has been a speaker at >20 international conferences.

FS 1.10| Olavi Reinsalu

Junior Research Fellow in Biomedical Technology, Faculty of Science and Technology, University of Tartu, Tartu,Estonia

Biography

Olavi Reinsalu received his diplomas for his bachelor's and master's degree in the curriculum of gene technology at the University of Tartu in 2012 and 2015, respectively. For the research of his master's thesis he studied virus-like particles carrying melanoma antigens. After graduation he worked for three years as a researcher in University of Tartu and Competence Center of Health Technologies developing a bovine fluorescence *in situ* hybridization technique as part of a R&D project for agricultural enterprises. Starting from fall 2018 he became a doctoral student in University of Tartu studying in the curriculum of technology and engineering specializing in biomedical technology. His PhD study is continuation of his research from master's and is also about extracellular vesicles and virus-like particles carrying melanoma antigens.

2.0. DAY 2: 1st of October 2022

DAY 2 Featured Speakers (FS)

FS 2.1| Richard Ferraro

Deputy Centre Head, Centre for Innate Immunity and Infectious Diseases, Research Group Head, Gastrointestinal Infection and Inflammation, Hudson Institute of Medical Research , Australia

Biography

Richard Ferraro is a Deputy Centre Head and Research Group Leader at the Hudson Institute of Medical Research. After completing his PhD at the University of NSW, in 1990, he took up a Postdoctoral Fellowship at the Institut Pasteur, Paris. In 1994, he was appointed to a tenured researcher position at the institute, where he subsequently developed an independent research group. In 2004, he returned to Australia to firstly take up a research/teaching academic position in the Department of Microbiology (Monash University) and then, in 2009, was recruited to his current position. His main research interests span the fields of *Helicobacter pylori*, bacterial membrane vesicles, NOD-like receptor proteins, and innate immunology. His research has translated to important outcomes in the areas of *H. pylori* pathogenesis, vaccine development and innate immunology. This research has been published in leading journals *i.e. Cell Host Microb.*, *Gastroenterol.*, *Immunity*, *Nat Rev Immunol.*, *Nat Immunol.* and *PNAS*. He hold positions on the Editorial Board of *Helicobacter*, the International Scientific Committee of "The International Workshop on Pathogenesis in *Helicobacter* infections", and abstract review panels for major international conferences in the fields of gastroenterology and *H. pylori* research.

FS 2.2| Una Riekstiņa

Professor, Department of Pharmacy , Faculty of Medicine, The University of Latvia, Riga, Latvia

Biography

Prof. Una Riekstiņa, the director of the Pharmacy bachelor's and master's study programmes at the Faculty of Medicine, the University of Latvia. She was nominated from Latvia to the European Medicines Agency's Committee of Advanced Therapies in 2014. Her research interests include mesenchymal stem/stromal cells, targeted drug delivery to the tumor microenvironment, and biological medicines such as advanced therapy medicinal products. In her presentation entitled "The EU regulatory framework for EV-based medicinal products", she will discuss the challenges of translating extracellular vesicle (EV) research into clinical applications, as well as the regulatory requirements for EV-based medicinal products.

FS 2.3| Diana Romenskaja

Junior Researcher, Centre for Innovative Medicine, Lithuania

Biography

Diana Romenskaja have a bioengineering bachelor's degree and medical biology master's degree. Currently she is junior researcher and a PhD student at Centre for Innovative Medicine. Since the beginning of her scientific journey, she has been working with microglial cells and extracellular vesicles isolated from oral mucosal stem cell supernatants. She focuses on research of interplay between extracellular vesicles and autophagy, lipid raft formation processes in human microglial cells.

FS 2.4| Lilite Sadovska

Researcher, Latvian Biomedical Research and Study center, Riga, Latvia

Biography

Lilite Sadovska is working as a researcher at the Latvian Biomedical Research and Study center she is currently reading for her PhD and studied Molecular Biology in the University of Latvia. Her current research works are focused on functional effects of cancer derived EVs on other tumor microenvironment cells, exercise induced EVs and their RNA content and functional effects, EVs RNAs as diagnostic or prognostic tools in cancer.

FS 2.5 | Artūrs Ābols

Head of the Lab-on-a-chip in the extracellular vesicle research group, Latvian Biomedical Research and Study Centre, Riga, Latvia

Biography

Arturs Abols is Early Career Investigator and head of Lab-on-a-chip in the extracellular vesicle research group at Latvian Biomedical research and study centre. The main goal of his group is to study potential applications and modifications of extracellular vesicles (EVs) from different sources, such as mesenchymal stem cells (MSC) and microbiota in cancer treatment using Lab-on-a-Chip (LoC) including Organs on Chip (OOC) technology. This interdisciplinary research direction stems from his postdoc in EV field as cancer biomarkers and recent collaboration with the Laboratory of prototyping from Institute of Solid-State Physics with expertise in microfluidics and biosensors. This research direction and collaboration also resulted with company CellboxLabs establishment, that focuses on PDMS (Polydimethylsiloxane) free organs on chip technology development, where Arturs Abols is cofounder and Chief Scientific Officer. He has PhD in molecular biology and more than 10 years of experience in cancer research and EV field.

FS 2.6 | Kasun Godakumara

Research Fellow, Institute of Veterinary Medicine and Animal Sciences, Estonian University of Life Sciences, Tartu, Estonia

Biography

Kasun Godakumara has started his research career in university of Peradeniya, Sri Lanka, where he studied hard tissue biology in osteoporosis conditions. He has earned his Bachelor's in Medical Laboratory Science and Master of Philosophy in Biotechnology from the University of Peradeniya in 2013 and 2016, respectively. After being employed as a laboratory analyst in the chemical pathology sector, he joined University of Tartu as a doctoral student in 2018. He was attached to the ERA chair of translational genomics (TransGeno) under the supervision of Prof. Alireza Fazeli. His studies are focused on extracellular vesicle based embryo maternal communication. Over time, He have gained expertise in numerous molecular biological, cell biological and bioinformatical technologies related to embryology and the biology of extracellular vesicles.

FS 2.7 | Emilija Šipailaitė

Researcher, Lithuanina University of Health Sciences, Kaunas, Lithuania

Biography

Emilija Šipailaitė work at Lithuanian University of Health Sciences. She has a Pharmacy Master's degree. She has carried out research in the cell culture laboratory for the last 3 years. During that period, she had a chance to work with many different cell cultures: human skin cells, stem cells and various cancerous cells. She has experience working with exosomes from stem cells and NT2 Parkinson's disease cybrids. Also, She took part in education programme "Basics to Work With Extracellular Vesicles" in the Institute of Biomedicine and Traslational medicine (MVBS.TK.013), 26 hours (1 ECTS). In addition, in the summer of 2021 I had an ERASMUS+ traineeship "Exosome signaling in bidirectional Microglia - Neuron communication" in University of Coimbra, Portugal. Recently, her field of interest is plant extracellular vesicles of plants and their effect to human cells.

FS 2.8 | Dovydas Gečys

Junior Researcher, Lithuanian University of Health Sciences, Kaunus, Lithuania

Biography

Dovydas Gecys is a junior researcher and a PhD student at Lithuanian University of Health Sciences. Currently he is working in Extracellular Vesicle research area with a focus to functionalize EVs for targeted drug delivery to glioblastoma cells. Additionally, he is working with biomarker research, gene engineering and epigenetics.

FS 2.9 | Getnet Midekessa

Research Fellow, Faculty of Biomedicine, University of Tartu, Estonia

Biography

Getnet Midekessa received his BSc in Applied Chemistry from Hawassa University, Ethiopia. Following graduation, he worked in the food complex and pharmaceutical industry in Ethiopia. Later he completed his master's studies in protein science and Biotechnology from the University of Oulu, Finland. During his master studies and post-graduation, he worked on projects involving protein characterization and Extracellular vesicles research at the Faculty of Biochemistry and Molecular Medicine at the University of Oulu. Getnet started his doctoral study in March 2018 in the Institute of Biomedicine and Translational Medicine, University of Tartu under the supervision of Prof. Fazeli. He is also working as a specialist at the Estonian University of Life sciences in the Institute of Veterinary Medicine and Animal Sciences. His research interests are focusing on the detection of surface extracellular vesicles using different biosensing technologies, as well as developing a novel diagnostic biomarker for different diseases

FS 2.10 | Cristina Bajo-Santos

PhD candidate, University of Latvia, Riga, Latvia

Biography

Cristina Bajo-Santos is currently a PhD candidate at University of Latvia. Prior to that, she studied BSc + MSc in Biology at University of Salamanca, Spain and later on she obtained her master by research in Biomedicine at University of Edinburgh, UK. She is currently studying the EV RNA content and its implications as potential biomakers for Prostate Cancer diagnosis and monitoring.

ABSTRACTS of the featured presentations

Day 01: 30th September 2022

AB1.1: Baltics Society of Extracellular Vesicle research potentials and capacities

Alireza Fazeli

Baltics Society of Extracellular Vesicle (BSEV) was created in 2022 with the aim of fostering Extracellular Vesicles (EV) research activities in Baltic Countries. The society currently has active members from all over the world including Estonia, Latvia and Lithuania.

BSEV, not even being one years old, thanks to the enthusiasm of researchers involved in EV research has managed to create its first research school and research conference. These are all positive signs and encouraging indications of BSEV founders' interest and enthusiasm to work and collaborate together. BSEV as mentioned in its founding constitution, will be an active society and will promote and support many different activities to advance research in the field of EVs in Baltic countries and beyond. During the presentation I will highlight many different strong points that BSEV can interact with other researchers in Baltic countries as well as fostering international collaborations between the researchers in Baltic countries and beyond.

AB1.2: Extracellular vesicles as a potent therapeutic tool against neurodegenerative diseases

Augustas Pivoriūnas

Extracellular vesicles (EVs) provide a potent tool for intercellular communication by acting as a miniature lipid membranous containers for wide array of signaling molecules. EVs have several advantages from a therapeutic perspective: (1) EVs are safer in comparison to cells, because of reduced risks associated with transplantation; (2) EVs are relatively simple, stable and controllable systems, being thus suitable for the large scale clinical manufacturing; (3) EVs can cross blood brain barrier and therefore can be effectively used for the treatment of different neurological conditions. We and others have demonstrated that EVs can be successfuly used as a potent therapies against Parkinson's disease (PD). During my talk I wil present our data about the therapeutic effects of the intranasal administration of EVs to the Parkinsonian rats. I will also discuss potential neuroprotective mechanisms of the EVs. Finally, I will talk about the challenges that we need to overcome in order to move EV therapies towards clinical application in humans.

AB1.3: Small non-coding RNA landscape of extracellular vesicles from an experimental model of equine osteoarthritis

Mandy Peffers

Joint tissues release extracellular vesicles (EVs) that potentially sustain joint homeostasis and contribute to osteoarthritis (OA) pathogenesis. EVs are putative novel therapeutics for OA, and transport biologically active molecules (including small non-coding RNAs (SNCRNAs)) between cells. This study identified altering SNCRNA cargo in EVs in OA which may act as early diagnostic markers and treatment targets.

OA was surgically induced in four skeletally mature Standardbred horses using an osteochondral fragment model in the left middle carpal joint. The right joint underwent sham surgery. Synovial fluid (SF)and plasma were obtained weekly throughout the 70-day study. EVs were isolated using size exclusion chromatography and characterised using nanoparticle tracking (Nanosight), and exosome fluorescence detection and tetraspanin phenotyping (Exoview). RNA was extracted from EVs derived from SF (sham and OA joints) and plasma collected at days 10, 35, 42, 49, 56, 63, and subjected to small RNA sequencing on a NovaSeq SP100 flow cell (Illumina).

Nanosight-derived EV characteristics of size and concentration were not significantly different following disease induction. The diameter of the temporal population of plasma and SF-derived exosomes changed significantly for both CD9 and CD81 following OA induction with significant temporal, and disease-related changes in CD63 and CD81 tetraspanin protein expressin in plasma and SF.

The differential expression temporally of seven microRNAs in plasma and synovial fluid-derived extracellular vesicles; eca-miR-451, eca-miR-25, eca-miR-215, eca-miR-92a, eca-miR-let-7c, eca-miR-486-5p, eca-miR-23a and four snoRNAs; U3, snord15, snord46, snord58 represent potential biomarkers for early osteoarthritis. Bioinformatics analysis of the

differentially expressed microRNAs in synovial fluid highlighted that in early osteoarthritis these related to the inhibition of cell cycle, cell cycle progression, DNA damage and cell proliferation, but increased cell viability and differentiation of stem cells.

Plasma and synovial fluid-derived extracellular vesicle small non-coding signatures have been established for the first time in a temporal model of osteoarthritis. These could serve as novel biomarkers for the evaluation of osteoarthritis progression or act as potential therapeutic targets.

AB1.4: Deciphering the physiological role of extracellular vesicles in milk: A One health approach

Marca Wauben

In mammals, milk is the first functional food and contains different components playing a role in the development of the gastrointestinal tract and immune system. One of these components are extracellular vesicles (EVs), i.e. cell-derived vesicles used for cell-cell communication. Comparative milk EV studies unveiled that miRNA and protein cargoes are highly conserved between species. The fact that milk EVs are stable and can cross epithelial borders has suggested that these EVs are involved in inter-organism communication. However, their role in developmental processes has been poorly studied. We explored the molecular mechanism of human mature milk EV-induced modulation of different cell types present in the gastrointestinal tract and found, by using a re-epithelialization gap closure assay, that milk EVs promote migration of oral epithelial cells. Functional integrative proteomic analysis unveiled hotspots of regulation in the p38 MAPK pathway targeted by milk EV proteins. Milk EVs also inhibited innate immune responses, e.g. agonist-induced endosomal Toll-like receptor 3 (TLR3) triggering of oral cavity epithelial cells, and adaptive immune responses, e.g. inhibition of αCD3/αCD28-induced CD4+ T cell activation, which could be linked to the presence of EV proteins targeting hotspots of regulation resulting in cell-cycle inhibition and mTOR stimulation. Interestingly, raw bovine milk-derived EVs could also inhibit human CD4+ T cell responses, indicating the conserved and cross-species activity of milk EVs. In conclusion, EVs are conserved bioactive structures in mature milk that can modulate canonical signal transduction pathways involved in key processes in the development of the epithelial barrier and the immune system of the new-born.

AB1.5: Blood-derived EVs as a source of biomarkers for early diagnostics of melanoma

Reet Kurg

Extracellular vesicles (EVs) have potential as new tumor markers that could be used as diagnostic and prognostic markers for early detection of melanoma. EVs were purified from the blood serum of melanoma patients using two methods—ultracentrifugation (UC) and PEG precipitation—and analyzed by mass spectrometry and immunoblot. We identified 585 unique proteins; 334 proteins were detected in PEG-precipitated samples and 515 in UC-purified EVs. EVs purified from patients varied in their size and concentration in different individuals. EVs obtained from stage II and III patients were, on average, smaller and more abundant than others. Detailed analysis of three potential biomarkers—SERPINA3, LGALS3BP, and gelsolin—revealed that the expression of SERPINA3 and LGALS3BP was higher in melanoma patients than healthy controls, while gelsolin exhibited higher expression in healthy controls. Our data suggest that all three proteins might have potential to be used as biomarkers, but a number of issues, such as purification of EVs, standardization, and validation of methods suitable for everyday clinical settings, still need to be addressed.

AB1.6: The role of Extracellular Vesicles in inflammatory signal transmission from the airway to the brain during viral infections

A. Jekabsone

Viral infections of the upper airways are the most common diseases affecting each individual. The infected cells produce extracellular vesicles (EVs) containing viral genetic material and inflammatory mediators. Small EVs such as exosomes remain stable in biofluids, penetrate well into the tissues, and easily cross biological barriers, including the blood-brain barrier. Therefore, they can transmit the inflammatory signal to the brain; however, the hypothesis has not yet been experimentally tested. The study aimed to determine whether virus mimicking sequence poly(I:C)-primed airway EVs enter the brain and, if yes, how they might affect the inflammatory status of the brain cells.Poly(I:C)-primed airway EVs entered the brain within an hour after intranasal delivery and localised primarily in microglial cells. The EVs internalised poly I:C molecules and caused inflammatory immunometabolic profile of microglial cells, including energy production switch from mitochondrial to glycolytic, generation of reactive oxygen species and inflammasome-related caspase-1 activation. The EVs significantly elevated the expression of inflammatory genes in cultured primary rat microglia, human microglial cells and the brain tissue of EV-treated mice. In conclusion, EVs from virus-infected airway cells might transmit viral particles and inflammatory signalling to the brain via microglial cells.

AB1.7: Spermatozoa, acts as an external cue and alters the cargo and production of the extracellular vesicles derived from oviductal epithelial cells

Qurat Ul Ain Reshi

The oviduct provides optimum physiological and biochemical milieu essential for successful fertilization as well as early embryo development and facilitates functional maturation of spermatozoa. Studies have showed that spermatozoa have the capability to alter gene expression in bovine oviductal epithelial cells (BOECs) remotely via secreted bio-active particles, thus acting as a cue to the oviduct prior to their arrival. However, very little attention has been paid to the question of whether spermatozoa could alter the cargo of extracellular vesicles (EV) derived from BOECs. Therefore, the aim of this study was to investigate the alterations in small non-coding RNAs in EV cargo derived from BOECs when these BOECs were incubated with spermatozoa in contact and non-contact co-culture models. After 4 hours of incubation the EVs were purified from the conditioned media, followed by small non-coding sequencing of the BOEC derived EVs. Our results revealed a distinct cargo in the form of mRNA, miRNA, tRNA and piRNA, present in EVs purified from contact and non-contact co-culture models when compared to the control. The pathway enrichment analysis revealed that EV mRNA from direct co-culture was associated with genes that were involved in the activation of multi-vesicular body (MVB) and microtubule pathways. Similarly, the EV mRNA derived from non-contact conditioned media, the genes were associated with suppression of immune system. Moreover, miR-100 and miR-99a-5p were found to be upregulated in contact co-culture models, the target genes of which regulate the phosphatidylinositol signalling system. The findings of this study suggest that spermatozoa, in contact as well as remotely, alter the EV cargos of female reproductive tract epithelial cells which might be playing an essential role in pre and post-fertilization events. These cellular events including inter-cellular communication via EVs are essential for a healthy pregnancy to occur.

AB1.8: Nanoparticles with surface display of transmembrane proteins as a tool for drug screening targeting G-protein coupled receptors

Sergei Kopanchuk

Naturally occurring extracellular bio-nanoparticles (be they viruses, microvesicles, exosomes, etc.) can be tailored to present a wide range of drug targets, including G protein-coupled receptors (GPCRs). These particles provide a novel source of complex membrane proteins that will be maintained in their native conformation in the lipid bilayer derived from cells in which GPCRs are potentially pharmacologically relevant. GPCRs share several common features, including the coupling of their signal transduction via G proteins. For mechanistic studies, it would be of great interest to have the possibility to enrich these particles, in addition to GPCRs, with G proteins and other modulators. For this purpose, we have developed a modified MultiBacMam technology that allows to express with a single baculovirus several proteins in the same mammalian cell with high efficiency. We detected a large amount of Frizzled 10 receptors on extracellular vesicles, which could be the result of the dispatching from filopodia where they are preconcentrated. To clarify this issue, we also developed a baculoviral library that coexpresses different markers together. As example, multicolor labeled CD9, CD63, CD81 and the plasma membrane markers. MultiBacMam technology also allows us to enrich the receptor fraction in the extracellular space through the production of Rous sarcoma virus-like particles or budded baculoviruses. For particle characterization and study of drug-receptor interactions on the particles, we used total internal reflection fluorescence microscopy with single molecule sensitivity.

AB1.9: Comprehensive characterization of RNA cargo in EVs from a longitudinal cohort of breast cancer patients undergoing neoadjuvant chemotherapy

Aiije Liine

Extracellular vesicles (EVs) are gaining increased attention as carriers of cancer-derived molecules for liquid biopsies. Here, we studied the dynamics of EV levels in the plasma of breast cancer (BC) patients undergoing neoadjuvant chemotherapy (NAC) and explored the relevance of their RNA cargo for the prediction of patients' response to the therapy. EVs were isolated from serial blood samples collected at the time of diagnosis, at the end of NAC, and 7 days, 6, and 12 months after the surgery from 32 patients with locally advanced BC, and 30 cancer-free healthy controls (HCs) and quantified by nanoparticle tracking analysis. The pre-treatment levels of EVs in BC patients were higher than in HCs, significantly increased during the NAC and surgery, and decreased to the levels found in HCs 6 months after surgery, thus showing that a substantial fraction of plasma EVs in BC patients are produced due to the disease processes and treatment. RNA sequencing analysis revealed that the changes in the EV levels were associated with the alterations in the proportions of various RNA biotypes in EVs. BC-derived biomarker candidates were identified among mRNAs, miRNAs, lncRNAs, snoRNAs, piRNAs, snRNAs, and tRFs. Several biomarker models for the detection and monitoring of BC and prediction of response to NAC were established. Furthermore, a number of RNAs that were induced by NAC in non-responders or patients with early disease progression were identified and warrant further functional studies on their role in chemoresistance and metastasis.

AB1.10: Cancer-testis antigen MAGE-A4 carrying EVs - tools for cancer theranostics

Olavi Reinsalu

Cancer-testis antigens (CTAs) are proteins that are normally expressed mainly in testis but aberrantly in various tumours. It has been shown that CTAs contribute to tumorigenic processes and, as antigens, these proteins are known to induce anticancer immune responses. MAGE-A4, a known CTA, is a soluble cytoplasmic or nuclear protein with a partially disordered structure. Although its exact cellular function has largely remained elusive, MAGE-A4 has been shown to have tumorigenic and antitumorigenic properties. We have discovered that MAGE-A4 is incorporated into virus-like particles and native extracellular vesicles (EVs) released by cells that express it. The artificially induced VLPs and natively emerged EVs expose MAGE-A4 to their outer surface, which is considered a fascinating phenomenon considering the intracellular localization of MAGE-A4. In the speech, MAGE-A4 carrying vesicles will be described showing their potential for anti-cancer therapeutic and diagnostic techniques.

Day 02: 1st October 2022

AB2.1: Bacterial extracellular vesicles transport bioactive products into host cells

Richard L. Ferrero

Extracellular vesicle (EV) formation is an evolutionarily conserved trait amongst members of all three domains of living organisms. EVs contain a diverse array of biologically active compounds and play a key role in cell-to-cell communication. The EVs released by bacteria (BEVs) contain biologically active products, such as proteins, cell wall components and toxins. Bacteria use BEVs as a means of delivering such factors into eukaryotic host cells, resulting in various biological responses in these cells. Although BEVs are highly effective at entering simple non-polarised cell monolayers, it is not known whether these nano-sized vesicles can penetrate an intact epithelial barrier and, potentially, disseminate their protein cargo to tissues. We have addressed this question using a cell culture model that reproduces the transepithelial resistance and apical-basolateral polarity of normal epithelium. We showed that *Helicobacter pylori* BEVs readily entered polarised epithelial cells but had no effect on the transepithelial resistance nor permeability of these monolayers. OMVs induced the basolateral secretion of the neutrophil chemoattractant, interleukin-8 (IL-8), and expression of human leukocyte antigen class I and II molecules. In exosomes isolated from the basolateral compartment of BEV-stimulated cells, we identified peptides derived from eight *H. pylori* proteins, of which seven are surface- or membrane-associated and are known to localise within BEVs. Collectively, the data show that BEVs can enter polarised epithelial cells and deliver their protein cargo to exosomes. We propose that these exosomes may directly or indirectly present antigen to immune cells and even transport bacterial proteins to other tissue sites.

AB2.2: The regulatory issues with EV-based advanced therapy medicinal products from the CAT perspective

Una Riekstiņa

The EU regulatory framework for EV-based medicinal products will be discussed. Particular interest will be given to discuss challenges of translating extracellular vesicle (EV) research into clinical applications, as well as the regulatory requirements for EV-based medicinal products.

AB2.3: Novel insights into the molecular mechanisms underlying the effects of extracellular vesicles on human microglial cells

Diana Romenskaja

Dysregulated microglial response is important for the development and propagation of neurological disorders and therefore targeting of neuroinflammatory microglia is considered as a novel therapeutic strategy. Extracellular vesicles (EVs) containing multiple proteins, RNAs, lipids and metabolites effectively suppress neuroinflammation and induce neuroprotective effects in different pathological conditions. Nevertheless, the mechanisms by which EVs regulate microglial responses remain largely unexplored. During my talk I will present our recent data about the effects of EVs derived from human dental pulp stem cells on the migration, phagocytic activity, autophagy and inflammatory response of human microglial cells. I will also show that EVs interfere with Toll-like receptor 4 signalling, promote lipid raft formation and initiate downstream signalling pathways through milk fat globule-epidermal growth factor VIII (MFG-E8) – αVβ3/αVβ5 integrin – dependent mechanisms.

AB2.4: RNA content and functional effects of exercise-induced EVs in cancer cells

Lilite Sadovska

Several studies have shown that regular exercise can decrease the risk of several types of cancer as well as decrease treatment side effects, increase the quality of life, prevent recurrence, and increase survival of cancer patients. Our studies have shown that at least partially these effects might be facilitated through EVs released into circulation by various tissue cells during exercise. First, we developed a rat model that had physically active and sedentary animals, and we analysed the circulating EV RNA content before and after exercise as well as in non-active animals and found 20 differentially expressed genes (DEGs) between pre and post samples, and 52 DEGs between active and sedentary rat samples. Further, we examined the effects of these exercise induced EVs on the growth of prostate cancer *in vivo*. We saw that the median tumour volume was reduced by 35% in rats that received RUN-EV injections (P=0.03) comparing to the ones that received Ctrl-EVs. We did not see any statistically significant effect on the number of metastases or metastasis's location between the groups. To address these questions also in humans, we have analysed the circulating EV RNA content of female life-long runners and are looking into their effects on breast cancer cells *in vitro*.

AB2.5: Mesenchymal stem cell-derived extracellular vesicles as a drug carrier in PDMS-free lung cancer-on-a-chip

Artūrs Ābols

Lung cancer is the most common cause of death with more than 50% lethal prognosis in the first year after diagnosis and the 5-year survival rate less than 18%. Although several treatments are available, they are usually not cancer cell-specific and often leave the patients struggling with unwanted side-effects. Studies of mesenchymal stem cell (MSC) extracellular vesicles (EV) suggest that they have tumour tropisms and can be used as drug carriers to become modern drug delivery systems to cancer cells. Lung cancer-on-a-chip (LCoC) systems are novel vascularised in vitro model system with liquid flow to mimic drug delivery trough circulation and study cancer tissue response. Currently available LCoC models are developed from PDMS (Polydimethylsiloxane), that is not suitable for drug testing since it absorbs small molecules. Therefore, we established new LCoC model from thermoplastics to study MSC derived EVs loaded with cisplatin in comparison to EVs without cisplatin and cisplatin alone. LCoC was established by using stable cell line A549 and commercial primary cell line - HPMEC (Human pulmonary microvascular endothelial cells). Cisplatin loaded MSC EVs were produced from immortalized commercial adipocyte derived MSC – ASC52-telo by growing them in media with nontoxic cisplatin concentration. Cisplatin loaded EVs were administrated within endothelial channel of LCoC and compared between MSC EVs without cisplatin, cisplatin without EV and negative control within previously optimized flow. Cell viability, biological barrier integrity and migration was evaluated by different assays. Preliminary results showed that cisplatin loaded EVs do not decrease biological barrier integrity and decreased A549 cell migration to endothelial channel suggesting, that cisplatin loaded EV could potentially decrease chemotherapy effect on endothelial cells, while final results will be presented at conference.

AB2.6: How important is the RNA cargo of Extracellular Vesicles in mediating their function?

Kasun Godakumara

One of the most critical steps in mammalian reproduction is Implantation. Embryos with impaired capacity of embryo-maternal cross talk are thought to have reduced potential for implantation. One agent of embryo-maternal communications are extracellular vesicles (EV). The mechanism used for EV based embryo-maternal cross talk remains elusive. One hypothesis put forward in this context is EV cargo when uptaken by the target cell is released to the cytoplasm and causes the required cascade of effects.

Since EVs are thought to be especially enriched in miRNA, they have a high possibility of being the actual agents of intercellular communication. There have been many reports of EVs carrying miRNA that can influence the target cells in a biologically relevant manner. EVs carrying the specific miRNA seem to induce the expected effect when supplemented to the target cells implying a logical connection between the EV derived miRNA and the effect on the target cell. In our experiments using *in vitro* embryo-maternal communication models, we have observed transcriptomic effects induced by trophoblast EVs on endometrial cells that can be attributed to trophoblast EV derived miRNA. However, the portion of transcriptomic changes that can be attributed to trophoblast EV derived miRNA was less than 10% of the overall transcriptomic change.

There are several significant facets to this seemingly perfect narrative of EV RNA based communications that is not widely discussed. Firstly, the amount of RNA carried in EVs are minute to the degree that a truly huge amount of EVs should be uptaken by the cells for a significant effect to be induced. Secondly, there is very little evidence that suggest any significant amount of EV cargo is actually released to the cytosol of the target cells, a

requirement for RNA based communication. Thirdly, some reports suggest the RNA carried in EVs are reported to be in a highly fragmented state, implying that the RNA might not be fully biologically active.

The final consensus on whether EV based RNA is a valid agent for intercellular communication is not reached. The way forward must be in experimentation that takes into account the quantity and the nature of RNA actually delivered by EVs and the fate of the RNA once the EVs are uptaken by the target cells.

AB2.7: Investigation of cucumber extracellular vesicles and their bioactivity on human skin cells

Emilija Šipailaitė

Plant-derived extracellular vesicles (EVs) are a novel research subject because of their potential to be used in improving and functionalisation of biopharmaceutical delivery. EVs are attractive delivery systems due to their ability to increase stability, solubility, and bioavailability of active agents of the plant. This study aimed to evaluate cucumber EV bioactivity on human skin cells. Fluorescence microscopy confirmed the internalisation of plant-derived EVs into the human skin cells after 24 hours. PrestoBlue™ metabolic cell assay revealed that EVs from cucumber fruits significantly increase proliferation of keratinocytes (HaCaT) and fibroblasts (HDF) after 48 hours. Moreover, the EVs shortened wound healing time in *in vitro* skin model. Therefore, EVs isolated from cucumber can be applied for development of regenerative cosmeceutical and medicinal products.

AB2.8: Internalisation of RGD-extracellular vesicles in glioblastoma cells

Dovydas Gečys

Glioblastoma multiforme (GBM) is the most common malignant type of tumour in central nervous system with one of the worst survival rates in the world. Despite available surgical and chemo/radio therapy treatments, most of the patients pass away within the first year of treatment. Poor outcome is caused by invasiveness, fast tumour recurrence and limited chemotherapy options [1,2]. Extracellular vesicles, a natural vehicle of bioactive materials, possess several essential attributes for drug delivery: ability to cross blood-brain barrier, low immunogenicity, specificity to the recipient cells and ability to be loaded with exogenous cargo [3,4]. It has been established that GBM cells have increased expression of transmembrane receptors called integrins which recognise tripeptide Arg-Gly-Asp (RGD) sequence [5]. Present study aims to investigate GBM internalisation efficacy of exosome-like particles modified with RGD peptide.

AB2.9: Evaluation of lipophilic dye labelling of extracellular vesicles using fluorescent nanoparticle tracking analysis

Getnet Midekessa

Different fluorescent molecules have been used to label EV membranes. A well-established fluorescent method involves maintaining the size of EVs after labelling. CellMask™ Green (CMG) dyes, which are lipophilic fluorescent dyes, are widely used to label plasma membranes of EVs. Using fluorescent NTA (*fl*-NTA), we investigated conditions that affect the optimal CMG labelling of EVs derived from human choriocarcinoma JAr cells and different biological fluids. The effect of CMG labelling on the size, concentration, and zeta potential (ZP) of JAr EVs purified using different methods, incubation temperatures, and detergent treatments was assessed. According to Fl-NTA analysis, the mean size of fluorescent nanoparticles (*fl*-NPs) decreased significantly with increasing concentrations of CMG dye ($p \leq 0.05$). Furthermore, *fl*-NPs derived from JAr cells with the lowest and highest dye concentrations showed a significant shift towards more and less negative ZP values, respectively ($p \leq 0.05$). A difference in the concentration of *fl*-NPs was observed between JAr EVs purified via size-exclusion chromatography (SEC) alone and those purified using SEC combined with tangential flow filtration. The proportion of *fl*-NPs in bovine follicular fluid and seminal-plasma derived EVs was higher and lower than that in cell-culture derived EVs, respectively. These findings suggest that CMG labelling may be useful for the detection and characterization of EVs using *fl*-NTA.

AB2.10: Plasma and Urinary Extracellular Vesicles as a source of RNA biomarkers in liquid biopsies of prostate cancer

Cristina Bajo-Santos

Extracellular vesicles (EVs) are released by virtually all cell types in the body and are present in various biofluids. Molecular cargo of EVs at least partially reflects the molecular composition of their cell of origin, however, what proportion of plasma and urinary EVs are derived from tumor tissue and to what extent their RNA cargo reflect the RNA content of cancer cells is unknown. In our study we characterized and compared the RNA cargo of plasma and urinary EVs collected before and after radical prostatectomy, and matched tumor and normal prostate tissues of prostate cancer (PCa) patients. We performed RNAseq, selected candidates through differential expression analysis and validated the results by ddPCR. Our study demonstrated that urine is significantly enriched with PC-derived RNAs as compared to plasma and suggested that mRNA fragments are the most abundant RNA biotype in EVs with a potential relevance for PCa detection and monitoring.

Poster presentations

PP1. Equine follicular fluid derived extracellular vesicles support cumulus expansion and alter cumulus cells transcriptome and viability; preliminary study

Julia Gabryś[1], Artur Gurgul[2], Tomasz Szmatoła[2], Barbara Kij-Mitka[1], Joanna Kochan1, Elżbieta Karnas[3], Monika Bugno-Poniewierska[1]

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Expansion of the cumulus cells (CC) surrounding the oocyte is essential for growth and maturation of a healthy oocyte, that can be fertilized and develop into an embryo. The follicular fluid (FF) is a rich source of numerous proteins, nucleic acids or extracellular vesicles (EVs), which are considered as mediators of intercellular communication within the follicle. Recent studies in cattle have shown an involvement of EVs in controlling cumulus expansion, but this effect was to date not observed in horses. To evaluate the effect of FF-derived EVs (ffEVs) on the equine CC expansion and viability cumulus-oocyte complexes (COCs) were matured in vitro (IVM) with EVs from small (<20 mm) equine ovarian follicles and were assessed by diameter measuring and staining. Additionally, CC were isolated after 12 hrs of IVM, after which RNA was extracted using modified TRI Reagent® Protocol and cDNA libraries were generated with QuantSeq 3′ mRNA-Seq Library Prep Kit. Transcriptome alterations of CC were investigated with next generation sequencing and bioinformatic analysis. COCs non treated with ffEVs were used as control. The obtained results confirm a significant influence of ffEVs on cumulus expansion in both compacted ($p<0.0001$) and expanded ($p<0.05$) COCs and indicate a divergent effect on the survival of CC during IVM. Despite the observed

differences in CC physiology, the transcriptome analysis demonstrated that the CC RNA profile is only slightly affected in the CC supplemented with ffEVs. Nevertheless, the differently expressed genes comprised ones connected with processes and functions (e.g. cellular processes, protein binding) which may be important for the properties of cumulus, its viability, expansion and hence the maturation of the oocyte. The study will be continued with more samples, different time points and EVs dosages to fully characterize the observed effect of ffEVs addition on CC physiology.

PP2: Human myometrial cells derived extracellular vesicles can stimulate the self-renewal of endometrial mesenchymal stromal/stem cells

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Human endometrium undergoes cycles of proliferation, differentiation and shedding during the female reproductive years. Endometrial mesenchymal stem-like cells (eMSC) contribute to this regenerative process. Myometrial cells have been identified as candidate niche cells of eMSC stimulating their self-renewal activity via paracrine mechanism. Whether other forms of intercellular communication, such as extracellular vesicles (EVs), may participate in stem cell maintenance in the human endometrium remains largely unknown. Full thickness endometrial tissues were obtained from women undergoing hysterectomy. After mechanical and enzymatic dissociation, endometrial stromal cells were purified from epithelial cells using EpCAM magnetic beads and leukocytes were removed using CD45 magnetic beads. EMSC- ($CD140b^+CD146^+$ cells) were then obtained using CD140b- and CD146-conjugated magnetic beads. To study how EVs mediate communication between eMSC and niche cells, myometrial derived EVs were isolated by ultracentrifugation. The EVs were

characterized by transmission electron microscopy and western blotting. The phenotypic expression and clonogenicity of eMSC cultured with myometrial EVs were determined by flow cytometry and colony formation, respectively. Our results demonstrated that myometrial EVs significantly enhanced the self-renewal and proliferation of eMSC. Specifically, the myometrial EVs carrying JAG1 activated Notch signaling in eMSC. The stimulatory effect of myometrial EVs on eMSC activities was abrogated when JAG1 was knockdown in the myometrial EVs. In summary, our finding revealed a new mechanism of the JAG1-mediated Notch signaling activation in the endometrial stem/stromal cell population.

PP3. Evaluation of three pre-treatment methods coupled with size-exclusion chromatography for the enrichment of extracellular vesicles from raw bovine milk

Madhusha Prasadani[1], Qurat Ul Ain Reshi [1, 2], Hanno Jaakson [3], Vallo Volke[2], Suranga Kodithuwakku [1], Alireza Fazeli [1,2,4]

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The extracellular vesicles (EVs) are membrane encapsulated nano-sized particles released by all types of cells and identified to be important in intercellular communications and signalling. The functional roles of EVs in normal homeostasis are highly appreciated and their involvement in pathophysiology of disease manifestation has also been greatly studied. High producing cows in general can undergo negative energy balance and subsequently develop insulin resistant (IR). Therefore, identifying non-invasive biomarkers during the early stages of this condition could improve

the health condition as well as the milk yield. Therefore, the present study was carried out to isolate and enrich the bovine milk EVs from fresh milk samples with the intention of using them as a tool to understand the early pathophysiology of IR in cows. Three different protocols of pre-treatments were adopted for fat and casein removal and size exclusion chromatography (SEC) was followed for EV enrichment. The first method was with starting volume of 50 ml of milk and two step filtrations, second method with 10 ml of starting volume and three step filtration and the third method with 10 ml starting volume, three step filtration and two SEC columns for the enrichment. According to the results obtained from the Nanoparticle Tracking Analysis, there was no significant differences ($p<0.05$) between the number of particles per unit volume. However, the method 2 showed higher enrichment plus high exosome percentages. The Transmission electron microscopic images depicted the presence of EVs, however high contaminants were also observed. Moreover, expression of CD 9 and CD 63 EV markers have confirmed the presence of EVs in the enriched fractions. Therefore, further analysis and investigations are warranted for the application of enriched EVs as a non-invasive diagnostic material.

PP4: The effect of trophoblast derived extracellular vesicles on Toll-Like Receptors expression in an endometrial cell line

Seyed Omidreza Mousavi[1], Kasun Godakumara[2], Suranga Kodithuwakku[1] and Alireza Fazeli[1,2,3]

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Interactions between the semi-allogenic embryo and the maternal cells during human embryo implantation is yet to be fully understood. Recently, different studies have shown that Toll like receptors (TLRs) mediated NFκB pathways may be involved in these interactions. Extracellular vesicles are being recognized as a new method of intercellular interactions. The present study was designed to decipher the immune related cross-talk between the embryo and the mother via TLRs and EVs. The abundance of each TLR (1 to 10) in receptive endometrial epithelial cells RL95-2, was determined with qPCR. The results demonstrated that TLR 4 and 8 expression levels were lower than the detection limit. However, the most abundant TLRs in this endometrial cell line were TLR 3 and 5 with 2×10^7 and 5.6×10^6 copies/10 ng input RNA, respectively. Hence, RL95-2 cells were treated with 10^9/ml of trophoblastic JAr cells derived EVs (T-EVs) at 70% confluency for 12, 24 and 48 hours, separately. EVs from non-trophoblast cell HEK293 were used as a negative control. Then the cells were harvested and the expression of TLR 3 and 5 in response to treatment with T-EVs were investigated. The transcriptional expression showed that T-EVs did not significantly affect the expression of these TLRs compared to the non-treatment group. Also, Treatment with HEK derived EVs did not affect the TLRs expression at transcript level. Further studies are needed to understand the interactions

at protein levels to delineate whether T-EVs can induce any immune reaction via TLRs pathways during the embryo implantation process.

PP5: Comparison of extracellular vesicles isolation methods from healthy individuals and diabetic patients' urine

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Worldwide around 6.7 million adults have died due to diabetes in 2021 alone. Diabetes causes severe complications such as neuropathy, nephropathy and retinopathy reducing patients' welfare as well as being a burden on the governmental and private healthcare budget. Novel and precise disease assessment methods are needed to prevent diabetes complications and death. Extracellular vesicles (EVs) are lipid-bound nano-sized particles found in all bodily fluids and have shown promise as biomarkers in various diseases. EVs from urine could be used as a marker for diabetes diagnostics and prognostics. We investigated urinary EVs isolation and purification methods for 6 diabetic patients' and healthy individuals' urine. Four protocols were compared, i.e., differential centrifugation with filtration and size-exclusion chromatography (SEC)(protocol 1), differential centrifugation with salt precipitation and SEC(protocol 2), differential centrifugation with protein organic solvent precipitation (PROSPR)(protocol 3) and differential centrifugation with PROSPR and SEC(protocol 4). We compared all the isolation methods for EV yield as determined by EV concentrations based on nanoparticle tracking analysis (NTA), EV purity as determined by protein

concentrations using Bradford assay and EVs physical characteristics assessed by transmission electron microscopy (TEM). In addition, we assessed the protocol adjustability to over-representation of protein contaminants in the initial urine sample from patients in case of proteinuric urine. EV concentrations (Mean ± SEM) showed presence of diverse particle concentrations between individuals and methods. Particle concentrations for protocol 4 (4.67E+08±3.05E+08) were significantly lower than protocol 1 (1.45E+10±3.43E+09) and protocol 2 (1.90E+10±7.12E+09) concentration. TEM imaging showed 30 to 100nm cup-shaped particles for protocols 1 and 2. Protocols 3 and 4 showed visibly fewer or no particles. Low protein contamination (<0.5mg/ml) was detected for all protocols. Our results confirmed that differential centrifugation with salt precipitation and SEC is the most effective methodology in the overall comparison of sample purity, EV yield and adjustability to urine protein concentrations and most suitable technology for EV isolation from urine. This method can be used for further studies to assess EVs potential role as diabetic biomarkers.

PP6: Subcellular Localization of Zinc Finger Protein 81; as a potential Extracellular Vesicle borne mediator of embryo maternal communication

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Zinc Finger protein 81 (*ZNF81*) gene encodes a protein that belongs to the zing finger family proteins and known as a potential transcription factor. Previously we have shown that trophoblast spheroid derived extracellular vesicles (EVs) can specifically down-regulate the ZNF-81 gene expression in receptive endometrial cells *in vitro*. However, the exact functional role or

subcellular localization of ZNF81 protein is yet to be understood in the endometrium. There is no clear report on ZNF81 subcellular localization so far in any other cell type as well. Therefore, the aim of this study was to detect the ZNF81 protein expression and localization in receptive endometrial epithelial cells and trophoblast cells *in vitro*. ZNF81 protein expression and localization in receptive endometrial analogue RL95-2 cells and trophoblast cell analogue JAr cells were analysed using immunofluorescent staining and confocal microscopy imaging. Then, RL95-2 cells were treated with JAr cell derived EVs for 24 hrs and ZNF81 protein localization was detected using immunofluorescence method. Based on our results, ZNF 81 protein mainly showed perinuclear cytoplasmic localization hinting to its possible role as a transcription factor. The localization was significant in perinuclear intracellular vesicles and weakly in cytoplasm in both cell types. JAr cells had high abundance of ZNF81 protein compared to RL95-2 cells. However, JAr cell derived extracellular vesicles did not cause a significant change in ZNF81 protein localization in RL95-2 cells. Further studies are required to decipher exact functional role of ZNF 81 protein both in receptive endometrium and trophoblast cells.

PP7: MicroRNA profile of extracellular vesicles isolated from the serum of piglets after exposure to PET microplastic

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Plastics and their products have become an integral part of our daily lives. They are used in household, agricultural, medical, pharmaceutical, and

engineering applications, and also serve as the main packaging for food transportation. When they enter the environment, they break down into small pieces called microplastics (MPs). One of the most common thermoplastic polymer resins in the polyester family is polyethylene terephthalate (PET). Exposure to microplastics has been reported to cause oxidative stress, cytotoxicity, and translocation to other tissues, while their permanent nature limits their removal from the organism, leading to chronic inflammation and increased cancer risk. MPs may also be involved in the increasing incidence of immune or neurodegenerative diseases. Here, we have shown that exposure to PET microplastics affects miRNA expression in serum exosomes. The experiment was performed on 8-week-old immature gilts. Animals were divided into two groups: a control group (n = 5) that received empty gelatin capsules orally and an experimental group (n = 5) that received a microplastics orally (0.1 g/swine/day in gelatin capsules) for 4 weeks. Extracellular vesicles (EVs) were isolated from serum by the ultracentrifuge method and verified by NTA and TEM. Small RNA-Seq analysis revealed 27 differentially expressed miRNA between the control group and the PET-treated group. Bioinformatics analysis showed that these miRNA might be involved in the regulation of insulin resistance, Th17 cell differentiation, and colorectal and pancreatic cancer signaling pathways. Our results showed that PET MPs impaired the cargo of EVs, suggesting their negative influence on the organism.

PP8: Extracellular DNA present in plasma small extracellular vesicles

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Extracellular DNA (ecDNA) is DNA found outside the cells and is accessible in many body fluids including blood plasma. In physiological conditions, most of ecDNA is cleared by deoxyribonucleases. EcDNA has also been found in exosomes that may protect it from degradation. This could contribute to increased concentrations of protected ecDNA. Whereas ecDNA is proinflammatory, it is important to find out whether DNA is protected from degradation in the circulation. The aim of this study was to investigate the presence and origin of DNA associated with exosomes and elucidate its localization on exosomes isolated from plasma. Exosomes were isolated from fresh healthy human plasma by ultracentrifugation with iodixanol-density gradient. The novelty of the current study lies in the investigation of localization and subcellular origin of the ecDNA associated with plasma exosomes as well as determining its approximate concentration. The cup-shaped exosomes were confirmed by transmission electron microscopy, with the highest concentration of particles in the size of 123 nm. The presence of exosomal markers CD9 and TSG101 was confirmed. We have confirmed the presence of mitochondrial DNA in all plasma exosomes, with the presence of nuclear DNA being more heterogenous. Our results show that while 60-75% of exosome-associated ecDNA is found on the outer membrane of exosomes, considerable amount is still protected from DNases inside vesicles. Whether the ratio of protected DNA changes in pathological states and how does it impact the induction

of inflammatory reaction remains to be elucidated. By confirming that predominantly mitochondrial DNA is protected from DNases treatment in exosomes, this can be potentially dangerous to the organism through pro-inflammatory function of exosomes.

PP9: Faecal Extracellular Vesicles: A novel non-invasive tool for understanding disease pathophysiology and diagnosis

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Extracellular vesicles (EVs) are heterogeneous and membrane-enclosed, nano-vesicles released from cells that mediate various physiological and pathological functions. The easy mobility of EVs through the different biological barriers allows the propagation of pathogenic proteins and genetic information throughout the body even in disease states. Since EVs are present in all biological fluids, there is great potential to develop EV-based disease diagnosis and monitoring tools. Feces is an easy-access and non-invasive clinical sample and can carry both host cells-derived, parasites and microbes derived EVs. In the current study, the main approach was to isolate and enrich the EVs from feces to develop a non-invasive disease diagnosis and monitoring tool. Cow faecal samples were directly collected from the rectum and suspended in PBS by vortexing. The suspension was subjected to isolate EVs by using differential centrifugation and size exclusion chromatography approach and characterized following

the guidelines provided by the International Society for Extracellular Vesicles. Twenty fractions of equal volume of elutes from each sample were collected and characterized by using nano particle tracking analysis (NTA), Bradford assay (BA) and transmission electron microscopy (TEM). The NTA results illustrated the presence of nanoparticles from fractions 4 to 13, and a maximum concentration of 2 x 10^{11} particles/ml was recorded in fractions number 6 and 7. The maximum free protein contamination was observed in fraction number 17, which was 0.13 mg/ml while particles enriched fractions (4 to 12) recorded very low contaminations. Interestingly, TEM results revealed that fractions 5,6 and 11 were contaminated with crystal/fiber-like structures. Thus, further studies are needed to increase the purity and characterization of faecal EVs. In conclusion, it seems EVs can be isolated from bovine faeces which can be used as non-invasive material in diagnostic and other applications.

PP10: Intranasally administered extracellular vesicles rescue memory and gait impairments in a time-dependent fashion in Parkinson's disease model rats

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Parkinson's disease (PD) is the second most common neurodegenerative disorder where progressive loss of dopaminergic (DA) neurons in the striatum and substantia nigra takes place, leading to cognitive and motor impairments. Current treatments are symptomatic and do not have a substantial impact on neither motor nor cognitive symptoms of PD. Extracellular vesicles (EVs) are particles capable of crossing the blood-brain barrier and transporting proteins, ribonucleic acids, lipids, and metabolites.

Intranasal administration of EVs is a promising strategy due to safety and stability. EVs rescued DA neurons from apoptotic death in vitro. In this study, we 1) determined whether intranasally administered EVs could reverse cognitive and motor dysfunction in PD model rats and 2) assessed whether these effects persist after termination of EV administration. We injected 6-hydroxydopamine (6-OHDA) into the medial forebrain bundle of adult male rats (0.02% ascorbic acid for controls) and initiated administration of EVs (for 17 consecutive days) to both 6-OHDA and controls a week later. Behavioural tests (apomorphine rotation, CatWalk™ gait and Morris water maze) were performed immediately after (short-term effects) and two weeks after (long-term effects) the termination of EV administration. Following euthanasia, striatal neuronal viability via Nissl body count and DA production via density of DA producing enzyme tyrosine hydroxylase (TH) were assessed in the striatum and substantia nigra (day 6 post-termination for short-term and day 20 post-termination for long-term groups). We showed that EVs time-dependently improved gait of PD model-rats for up to 10 days after EV treatment was stopped. However, EVs provided shorter memory enhancement – for up to 6 days post-treatment. Behavioural effects coincided with short-term increase in TH density in the striatum and substantia nigra and preserved striatal neuronal viability. We suggest that the lifespan of intranasal EVs is an important determinant of their effects in the rat model of PD.

PP11: Potato (*solanum tuberosum*) peel and root as potential sources of extracellular vesicles, a step towards the valorization concept

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Extracellular vesicles (EVs), play a crucial role in mammalian cell-to-cell communication, by transporting components such as proteins and small RNAs. Similarly, plants are also believed to be producing EVs under various circumstances. Herein, we attempted to purify and characterize vesicle-like structures separately, from initial sampling solutions containing root exudates of potato (*Solanum tuberosum*, cultivar: Laura) plants grown hydroponically and apoplastic wash collected from potato peels of the same cultivar, by a vacuum infiltration method using a vesicle isolation buffer (VIB: 20 mM, 2 mM $CaCl_2$, and 0.1 M NaCl, pH 6) following established protocols. The purification process was a combination of differential centrifugation steps (500g, 3000g, 10 000g for 15 minutes per each force respectively) and size exclusion chromatography, where 20 purified fractions (500 µL each) of the samples were collected for further examination. Biophysical and chemical analyses, was conducted on these EVs using Bradford Protein assay, Nanoparticle tracking, Flourescent Nanoparticle tracking, and transmission electron microscopy. The results showed that the size of the released EVs range (75-350 nm) and (70 – 320 nm) in potato roots and potato peels respectively and with particle concentrations of (7.0×10^9 particles/ mL) and (2.0×10^{10} particles/ mL) similarly, in potato roots and peels. We believe that, ultimately, the findings of this study will address the prevailing void in the field of studies related

to the characterization of plant EVs to a certain extent while tapping the potential of agri-food wastes and by-products as a cost-effective source for the mass production of EVs, which will eventually direct towards the sustainable use of renewable bioresources into high-value-added functional products under the concept of "valorization".

PP12: Anaerobic microbiota-derived extracellular vesicle research by PDMS-free gut on a chip

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The gut microbiota has a critical role in human health and are involved in all physiological processes. Bacterial derived extracellular vesicles (BEV) play a significant role in it. Currently, the study of the mechanism of human gut microbiota communication with host cells is complicated and research methods for these processes are limited. One of the promising model system for researching these processes is the gut-on-chip (GoC) platform. Therefore, the aim of our research is to study BEV RNA content of cancer patient microbiota, which can enter from lumen to circulation by applying GoC devices. To that end, we have currently developed a GoC device suitable for anaerobic microbiota cultivation. Next, we successfully optimised anaerobic microbiota isolation from human stool samples confirmed by metagenome sequencing and co-cultivation within GoC functionalised with stable cell lines. BEV from gut lumen and those that can pass through gut-endothelial barrier were collected and are currently analysed.

PP13: PDMS–free microfluidic device testing for extracellular vesicle separation

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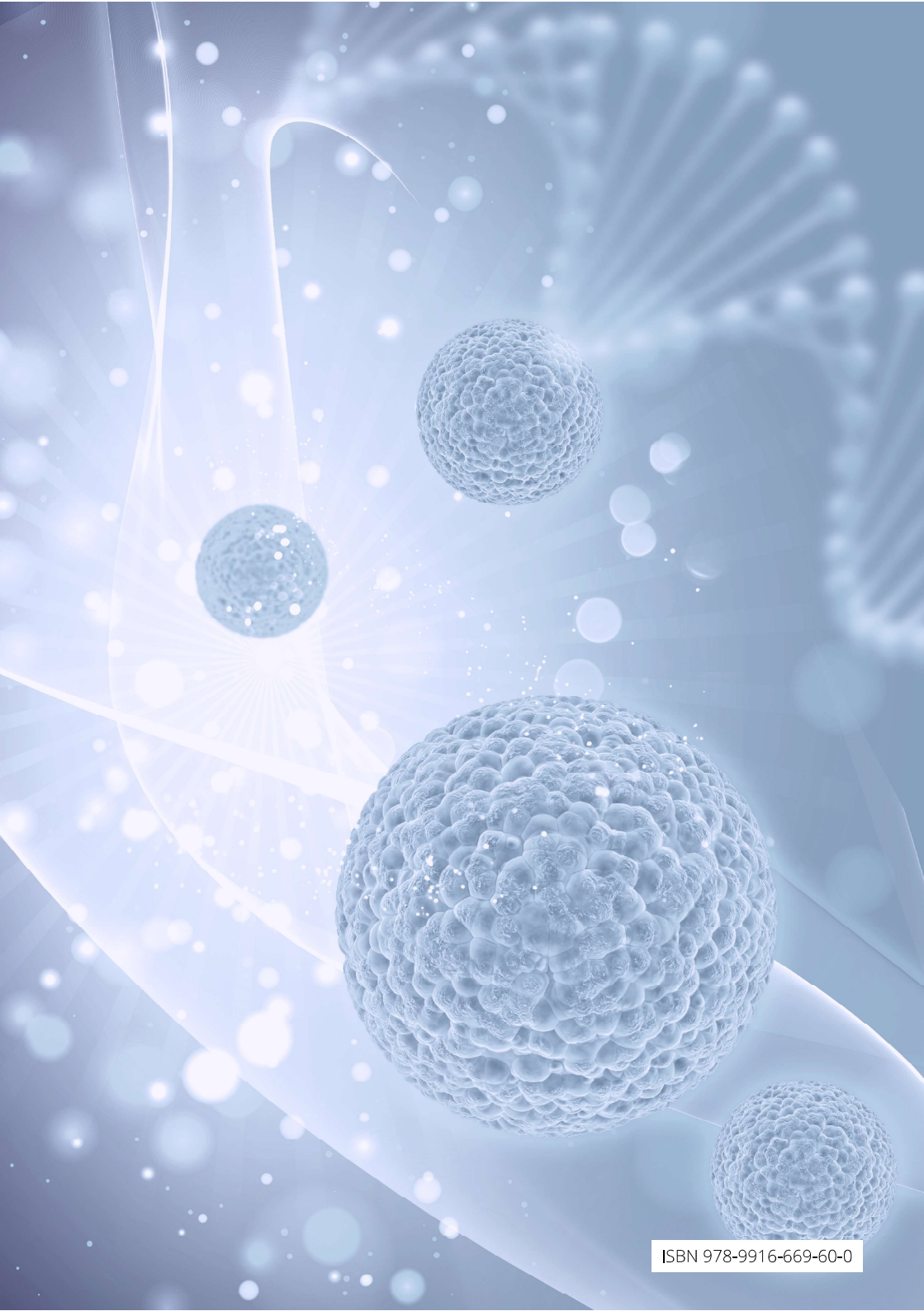
Current typical extracellular vesicle (EV) isolation methods have shown various limitations with purity, functionality and recovery efficiency which limits the reliability of the final product. More recent methods for EV isolation have come from the field of microfluidics, to solve current issues of EV isolation such as recovery efficiency, purity, and reproducibility. However, most such systems use PDMS (Polydimethylsiloxane) based polymer devices, that is not usable for mass manufacturing and have high hydrophobic small molecule absorption. Therefore, new polymer devices for EV isolation are necessary.

Two sets of microchannel devices, PDMS and OSTE (Off-stoichiometry thiol-ene polymer) were manufactured and optimised for sample flow through. Afterwards, both devices were tested for EV absorption.

Our results have shown that OSTE devices have superior characteristics for EV isolation in comparison to PDMS where the EV recovery for OSTE is about 85% whereas PDMS devices only had a recovery of 60%.



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