Oral with Poster Session 2
Chair: Francesc Borras
Location: Room 5

15:30–16:30

OWP2.01 = PS09.14

Isolation and phenotype characterization of microvesicle subpopulations from mixed cells in an *in vitro* model of lung microvascular injury

Nikhil Tirlapur; Kieran P. O'Dea; Michael Wilson; Masao Takata

Section of Anaesthetics, Pain Medicine and Intensive Care, Faculty of Medicine, Imperial College London, Chelsea and Westminster Hospital, London, United Kingdom, London, United Kingdom

Background: Methods to isolate microvesicle (MV) subpopulations derived from a mixed parent cell population, while preserving MV biological function, are not clearly established. We present a novel method of isolating endothelial- and monocyte-derived MVs from an *in vitro* model of lung microvascular injury using immunomagnetic bead separation.
Methods: Human lung microvascular endothelial cells were grown to confluence on flexible-bottomed plates. Primary human monocytes were incubated for 2 h with pre-activated endothelial cells (LPS 20 ng/ml, 24 h). Cells then underwent cyclic stretching for 16 h to model pulmonary microvascular injury were seen clinically in ventilator-induced lung injury. Culture media were harvested and underwent differential centrifugation to isolate MVs. Separation of MV subpopulations was performed by negative immunomagnetic bead separation, using beads coated either with anti-CD146 (binding endothelial-derived MVs) or with anti-CD11b (binding monocyte-derived MVs). Phenotypes of isolated MV subpopulations were confirmed by flow cytometry, and their biological function tested by MV (1×10^6) incubation with human umbilical vein endothelial cells (HUVECs) for 6 h, followed by flow cytometric analysis of their surface activation markers (E-selectin/ICAM-1/VCAM-1).
Results: Endothelial- and monocyte-derived MV subpopulations were successfully separated in our model, with >95% purity, negligible contamination with other MV subtypes, and recovery yield of 80–95% for endothelial-derived (CD146+ve) MVs and 70–85% for monocyte-derived (CD11b/CD45+ve) MVs. Monocyte-derived MVs, but not endothelial-derived MVs, induced significant HUVEC activation.
Summary/conclusion: Negative immunomagnetic bead separation provided efficient isolation of mixed MV subpopulations, preserving their individual phenotypes and biological function while maintaining reasonable recovery and purity. This methodology may be beneficial for functional analysis of individual MV subpopulations in samples from other *in vitro* models or *in vivo*/clinical samples.
Funding: Medical Research Council.

OWP2.02 = PS05.01

Detection and characterization of different neuronal and glial populations of exosomes by surface plasmon resonance imaging

Silvia Picciolini[1]; Alice Gualerzi[1]; Andrea Sguassero[1]; Furio Gramatica[1]; Massimo Masserini[2]; Marzia Bedoni[1]

[1]Laboratory of Nanomedicine and Clinical Biophotonics (LABION), IRCCS Fondazione Don Carlo Gnocchi, Milan, Italy; [2]Nanomedicine Center NANOMIB, School of Medicine and Surgery, University of Milano-Bicocca, Monza, Italy

Background: The use of exosomes for diagnostic and disease monitoring purposes is becoming particularly appealing, considering that the pathological status greatly affects the exosomes content. Moreover, brain-derived exosomes present in blood plasma could be seen as a direct read-out of the condition of the CNS and can thus be studied as peripheral biomarkers of neurological disorders. Inspired by remarkable development of plasmonic biosensors having the ability to detect exosomes, we have designed an antibody array using surface plasmon resonance imaging (SPRi) with the aims to detect CNS-derived exosomes present in human plasma and to characterize them according to the presence and the relative amount of membrane molecules.
Methods: Exosomes were isolated from plasma of healthy volunteers by size-exclusion chromatography and characterized by nanoparticles tracking analysis, transmission electron microscopy, western blot and a nanoplasmonic assay to check the sample purity. The SPRi array was optimized for the detection of exosomes subpopulations, by using a suitable surface chemistry and specific antibodies for each class of vesicle to be detected.
Results: Exosomes were detected and adsorbed on the SPRi chip, demonstrating the possibility to simultaneously distinguish exosomes derived specifically from neurons (Ephrin), microglia (IB4), astrocytes (GLAST) and oligodendrocytes (PLP) using the multiplexing SPRi approach. Moreover, the presence and relative amount of another membrane constituent (GM1) were then evaluated using a sandwich approach, showing a different composition of exosomes according to their cellular origin.
Summary/conclusion: SPRi can be used to discriminate the neuronal and the different glial populations of exosomes circulating in the peripheral blood and to perform their concomitant characterization. The optimized SPRi biosensor represents a promising platform for the characterization of exosomes involved in neurodegenerative and cerebrovascular diseases and for their possible use as clinical biomarkers.
Funding: Italian Ministry of Health, Ricerca corrente 2017–2018.

OWP2.03 = PS04.03

Microscale electrophoretic separations of exosomes

Yuliya Shakalisava; Delaram Zahouri; Roy Kreekel; Thomas Hankemeier

Leiden Academic Center for Drug Research, Leiden University, Leiden, The Netherlands

Background: Exosomes have gathered interest due to their diagnostic and therapeutic potential. They are present in blood, urine and saliva, which make them an appealing resource for non-invasive etiological and diagnostic research. Undoubtedly, size and optical properties are the most studied, which is reflected in the current isolation methods dominating the research field. Our research makes a contribution to further investigation of electrophoretic properties of exosomes. For the first time we report a microscale separation method capillary electrophoresis (CE) for characterisation of exosomes. The aim was to further explore electrophoretic behaviour of exosomes and investigate the electrophoretic signatures of exosomes in CE format.
Methods: CE was employed to study the electrophoretic migration of standards of exosomes in the narrow bore capillary under the electric field. Laser-induced fluorescent detector was used and different fluorescent markers were investigated for labelling of exosomes. Capillary zone electrophoresis (CZE) and capillary isotachophoresis(cITP) modes of CE were used in this study. To improve the resolution of exosomal fractions in cITP mode, various spacer compounds were investigated. The method was applied to the human exosomes samples.
Results: The multiple zones of exosomes can be seen in the electropherogram of exosomes standards. These indicate the subpopulations of exosomes within the commercial sample of purified exosomes. These subpopulations show differences in their electrophoretic mobility which are based on their size and charge properties. Different fluorescent markers provided an informative insight into the migration of different fractions of