Mammalian cell exosomes and other microvesicles from cells and conditioned medium

1. Introduction

There are three areas of investigation where iodixanol gradients have been widely used in studies of exocytosis and exocytotic vesicles.

1. The control and organization of membrane trafficking within the cells that permits the movement of vesicles to, and ultimately their fusion with, the plasma membrane or a specific plasma membrane domain. This is covered in OptiPrep™ Mini-Review MS12 and OptiPrep™ Application Sheet S45
2. The analysis of microvesicles that are expressed from the surface of cells is very widely researched and often involves separation from virus particles in the conditioned medium from virus-expressing cells. This Mini-Review provides a full bibliography of papers reporting these fractionations. A detailed methodology is provided in Application Sheet S61
3. A third associated area of investigation is the isolation and study of extracellular vesicles (EVs) from Gram-positive bacteria and fungi, and of outer membrane vesicles (OMVs) from Gram-negative bacteria. The latter in particular are widely researched and have been shown to be important in the transfer of virulence factors and the initiation of immune and inflammatory responses in host cells. This is covered in Mini-Review MS14 and Application Sheet S60

- Other Mini-Reviews or OptiPrep™ Application Sheets can be accessed on the flash drive or from the following website: www.axis-shield-density-gradient-media.com then click on "Mini-Reviews" or "Methodology". On the flash drive return to "Mini-Reviews" or "Application Sheets" to access other files.

2. Methodological summary

Various forms of pre-gradient processing are employed, during which intact cells and aggregated material in the culture medium are mostly removed and the exosomes or microvesicles concentrated. This is covered in much greater detail in Application Sheet S61

To minimize contamination of exosomes from the culture medium and or serum these solutions are either ultracentrifuged or filtered prior to contact with the cell monolayer. Occasionally serum-free medium is used.

Post-culture, cells and other large particles are first removed from the conditioned medium (CM) by differential centrifugation (**clarification step**). Sometimes a single low-speed centrifugation is used, more often two or three steps (e.g. 300 *g* and 5,000 *g*), usually for 10-15 min. Omission of the first step may lead to entrapment and loss of small vesicles into aggregates of rapidly-sedimenting larger particles at the higher *g*-force. Filtration is also used to remove larger contaminants: this is commonly performed using a 0.20 or 0.22 μm syringe filter, occasionally a smaller (0.1μm) or larger-pore (0.45μm) may be used. Filtration is usually used in combination with differential centrifugation, although it may be the only pre-gradient treatment.

Concentration of exosomes and other vesicles from the clarified CM usually involves pelleting 100-150,000 *g* for 1-2 h before resuspending in a suitable buffered medium for application to the iodixanol gradient; although there are variations to this strategy (see Application Sheet S61) 12,000 *g*, 70,000 *g* and 110,000 *g*. Particularly large volumes of CM may be treated to a preliminary concentration using centrifugal ultrafiltration (5 kDa-100 kDa cut-off), to reduce the total volume prior to ultracentrifugation. Occasionally a discontinuous sucrose gradient may be used as part of the concentration process.

Purification of exoxomes has been successfully executed in the following types of iodixanol gradient:

1. Top-loaded sedimentation velocity iodixanol gradients, normally centrifuged for 1.5-2 h. The gradients, although often constructed from multiple layers (i.e. discontinuous) the density interval of only 1.2% (w/v) iodixanol is so small that the 6-18% (w/v) iodixanol gradient is essentially continuous.
2. Bottom-loaded discontinuous gradients with centrifugation times of 2-3 h.

- Bottom-loaded or top-loaded continuous gradients; centrifuged usually at 100-200,000 *g* for 16-21 h; the vesicles are banded according to their buoyant density.

3. Self-generated gradients: usually run in vertical or near-vertical rotors at approx. 350,000 *g* for 2-3 h. The method, as with any self-generated gradient separation, has the advantage of being both simple to set up and capable of producing a very reproducible density profile.

- For full details of methodology see Application Sheet S61**.**

- **A full bibliography of published research papers is given in Section 3.**
- **Section 4 is devoted to the electroporation of exosomes.**
- **Reviews of methodology, exosome function and clinical application are listed in Section 5.**

3. Analytical studies on exosomes purified in iodixanol gradients – a bibliography

References are listed alphabetically (a) according to **tissue/cell type** and/or occasionally research topic and (b) **first author.** Research topic key words are highlighted in blue in the titles

Adipose tissue

Ying, W., Riopel, M., Bandyopadhyay, G., Dong, Y., Birmingham, A., Seo, J.B., Ofrecio, J.M., Wollam, J. et al (2017) *Adipose tissue macrophage-derived exosomal miRNAs can modulate in vivo and in vitro insulin sensitivity* Cell, 171, 372–384

Amoeboid cancer cells

Sobreiro, M.R., Chen, J-F., Morley, S., You, S., Steadman, K., Gill, N.K., Chu, G. C-Y., Chung, L.W.K., Tanaka, H. et al (2017) *Amoeboid cancer cells shed extracellular vesicles enriched with nuclear derived material* J. Extracell. Vesicles Abstr. PT01.04

Astrocytes/astrocytoma cells

Dominkuš, P.P., Ferdin, J., Plemenitaš, A., Peterlin, B.M. and Lenassi, M. (2017) *Nef is secreted in exosomes from Nef.GFP-expressing and HIV-1-infected human astrocytes* J. Neurovirol. **23**, 713–724

Rahimian, P. and He, J.J. (2016) *Exosome-associated release, uptake, and neurotoxicity of HIV-1 Tat protein* J. Neurovirol., **22**, 774–788

BHK cells

Hammarstedt, M. and Garoff, H. (2004) *Passive and active inclusion of host proteins in human immuno-deficiency virus type 1 Gag particles during budding at the plasma membrane* J. Virol., **78**, 5686-5697

Blood – see Plasma

Bone marrow mast cells

Kormelink, T.G., Arkesteijn, G.J.A., Nauwelaers, F.A., van den Engh, G., Nolte-'t Hoen, E.N.M. and Wauben, M.H.M. (2016) *Prerequisites for the analysis and sorting of extracellular vesicle subpopulations by high-resolution flow cytometry* Cytometry Part A, **89A**, 135-147

Bone marrow mesenchymal stromal cells

Bruno, S., Tapparo, M., Collino, F., Chiabotto, G., Deregibus, M.C., Lindoso, R.S., Neri, F., Kholia, S. et al (2017) *Renal regenerative potential of different extracellular vesicle populations derived from bone marrow mesenchymal stromal cells* Tissue Eng. Part A, **23**, 1262-1273

Brain tumour

Graner, M.W., Alzate, O., Dechkovskaia, A.M., Keene, J.D., Sampson, J.H., Mitchell, D.A. and Bigner, D.D. (2009) *Proteomic and immunologic analyses of brain tumor exosomes* FASEB J., **23**, 1541–1557

Breast epithelial and carcinoma cells

Baietti, M.F., Zhang, Z., Mortier, E., Melchior, A., Degeest, G., Geeraerts, A., Ivarsson, Y., Depoortere, F. et al (2012) *Syndecan-syntenin-ALIX regulates the biogenesis of exosomes* Nat. Cell Biol., **14**, 677-685
Clark, D.J., Fondrie, W.E., Liao, Z., Hanson, P.I., Fulton, A., Mao, L. and Yang, A.J. (2015) *Redefining the breast cancer exosome proteome by tandem mass tag quantitative proteomics and multivariate cluster analysis* Anal. Chem., **87**, 10462−10469
Lee, J-E., Moon, P-G., Cho, Y-E., Kim, Y-B., Kim, I-S., Park, H. and Baek, M-C. (2016) *Identification of EDIL3 on extracellular vesicles involved in breast cancer cell invasion* J. Proteom., **131**, 17–28
Thompson, C.A., Purushothaman, A., Ramani, V.C., Vlodavsky, I. and Sanderson, R.D. (2013) *Heparanase regulates secretion, composition, and function of tumor cell-derived exosomes* J. Biol. Chem., **288**, 10093–10099
Vergauwen, G., Dhondt, B., Van Deun, J., De Smedt, E., Berx, G., Timmerman, E., Gevaert, K., Miinalainen, I. et al (2017) *Confounding factors of ultrafiltration and protein analysis in extracellular vesicle research* Sci. Rep., **7**: 2704
Vergauwen, G., Dhondt, B., Van Deun, J., Timmerman, E., Gevaert, K., Braems, G., Van den Broecke, R. et al (2017) *Confounding factors in extracellular vesicle ultrafiltration and protein analysis* J. Extracell. Vesicles Abstr. OT7.02
Yang, Z., Xie, J., Zhu, J., Kang, C., Chiang, C., Wang, X., Wang, X., Kuang, T., Chen, F. et al (2016) *Functional exosome-mimic for delivery of siRNA to cancer: in vitro and in vivo evaluation* J. Control. Release, **243**, 160-171

Cerebro-spinal fluid

Hartmann, A., Altmeppen, H., Krasemann, S. and Glatzel, M. (2017) *Exosomes in prion diseases* In Prion Diseases, Neuromethods, **129,** (ed. Liberski, P.P.), © Springer Science+Business Media LLC, pp 197-207

Chloride intracellular channel protein 4 (CLIC4)

Sanchez, V.C., Craig-Lucas, A., Wei, B-R., Shukla, A., Read, A., Lou, J., Simpson, M., Hunter, K. and Yuspa, S. (2017) *Chloride intracellular channel protein 4 (CLIC4) is a serological cancer biomarker released from tumour epithelial cells via extracellular vesicles* J. Extracell. Vesicles Abstr. PF01.12

Cholangiocarcinoma cells

Khoontawad, J., Pairojkul, C., Rucksaken, R., Pinlaor, P., Wongkham, C., Yongvanit, P., Pugkhem, A., Jones, A., Plieskatt, J. et al (2017) *Differential protein expression marks the transition from infection with Opisthorchis viverrini to cholangiocarcinoma* Mol. Cell. Proteomics, **16**, 911-923

Colon/colorectal carcinoma cells (see also "Gastrointestinal cancers")

Belov, L., Hallal, S., Matic, K., Zhou, J., Wissmueller, S., Ahmed, N., Tanjil, S., Mulligan, S.P. et al (2017) *Surface profiling of extracellular vesicles from plasma or ascites fluid using DotScan antibody microarrays* In Serum/Plasma Proteomics: Methods and Protocols, Methods Mol. Biol., **1619**, (ed. Greening, D.W. and Simpson, R.J.) Springer Science+Business Media, LLC, pp 263-301
Choi, D-S., Park, J.O., Jang, C.S., Yoon, Y.J., Jung, J.W., Choi, D-Y., Kim, J-W., Kang, J.S., Park, J., Hwang, D. et al (2011) *Proteomic analysis of microvesicles derived from human colorectal cancer ascites* Proteomics, **11**, 2745–2751
Choi, D-S. and Gho, Y.S. (2015) *Isolation of extracellular vesicles for proteomic profiling* In Methods in Mol. Biol., **1295**, Proteomic Profiling: Methods and Protocols, (ed. Posch, A.) Springer Science+Business Media New York pp. 167-177
De Wit, M., Fijneman, R.J.A., Verheul, H.M.W., Meijer, G.A., and Jimenez, C.R. (2013) *Proteomics in colorectal cancer translational research: Biomarker discovery for clinical applications* Clin. Biochem., **46**, 466–479

Greening, D.W., Xu, R., Ji, H., Tauro, B.J. and Simpson, R.J. (2015) *A protocol for exosome isolation and characterization: evaluation of ultracentrifugation, density-gradient separation, and immunoaffinity capture methods* In Proteomic Profiling: Methods and Protocols, Methods in Mol. Biol., **1295** (ed. Posch, A.) Springer Science+Business Media New York pp 179-209
Hong, B.S., Cho, J-H., Kim, H., Choi, E-J., Rho, S., Kim, J., Kim, J.H., Choi, D-S., Kim. Y-K., Hwang, D. and Gho, Y.S. (2009) *Colorectal cancer cell-derived microvesicles are enriched in cell cycle-related mRNAs that promote proliferation of endothelial cells* BMC Genomics, **10**:556
Jang, S.C., Kim, O.Y., Yoon, C.M., Choi, D-S., Roh, T-Y., Park, J., Nilsson, J., Lotvall, J., Kim, Y-K. and Gho, Y.S. (2013) *Bioinspired exosome-mimetic nanovesicles for targeted delivery of chemotherapeutics to malignant tumors* ACS Nano, **7**, 7698–7710
Ji, H., Greening, D.W., Barnes, T.W., Lim, J.W., Tauro, B.J., Rai, A., Xu, R., Adda, C., Mathivanan, S. et al (2013) *Proteome profiling of exosomes derived from human primary and metastatic colorectal cancer cells reveal differential expression of key metastatic factors and signal transduction components* Proteomics, **13**, 1672–1686
Kim, S.B., Kim, H.R., Park, M.C., Cho, S., Goughnour, P.C., Han, D., Yoon, I., Kim, JH, Kang, T. et al (2017) *Caspase-8 controls the secretion of inflammatory lysyl-tRNA synthetase in exosomes from cancer cells* J. Cell Biol., **216**, 2201–2216
Mathivanan, S., Lim, J.W.E., Tauro, B.J., Ji, H., Moritz, R.L. and Simpson, R.J. (2010) *Proteomics analysis of A33 immunoaffinity purified exosomes released from the human colon tumor cell line LIM1215 reveals a tissue-specific protein signature* Mol. Cell. Proteomics, **9**, 197–208
McKenzie, A.J., Hoshino, D., Hong, N.H., Cha, D.J., Franklin, J.L., Coffey, R.J., Patton, J.G. and Weaver, A.M. (2016) *KRAS-MEK signaling controls Ago2 sorting into exosomes* Cell Rep., **15**, 978–987
Mertens, I., Castiglia, M., Carreca, A.P., Baggertman, G., Peeters, M., Pauwels, P. and Rolfo, C. (2014) *Exosome analysis in cancer patients: From the preclinical towards the clinical application: Trial design* Eur. J. Cancer, **50**, Suppl. 6, 96
Shinohara, H., Kuranaga, Y., Kumazaki, M., Sugito, N., Yoshikawa, Y., Takai, T., Taniguchi, K., Ito, Y. and Akao, Y. (2017) *Regulated polarization of tumor-associated macrophages by miR-145 via colorectal cancer–derived extracellular vesicles* J. Immunol., **199**, 1505–1515
Tauro, B.J., Greening, D.W., Mathias, R.A., Ji, H., Mathivanan, S., Scott, A.M. and Simpson, R.J. (2012) *Comparison of ultracentrifugation, density gradient separation, and immunoaffinity capture methods for isolating human colon cancer cell line LIM1863-derived exosomes* Methods, **56**, 293–304
Xu, R., Greening, D.W,m Rai, A., Ji, H. and Simpson, R.J. (2015) *Highly-purified exosomes and shed microvesicles isolated from the human colon cancer cell line LIM1863 by sequential centrifugal ultrafiltration are biochemically and functionally distinct* Methods, **87**, 11–25
Xu, R., Simpson, R.J. and Greening, D.W. (2017) *A protocol for isolation and proteomic characterization of distinct extracelluar vesicle subtypes by sequential centrifugal ultrafiltration* In Exosomes and Microvesicles Methods and Protocols: Methods Mol. Biol., **1545** (ed. Hill, A.F.), Springer Science+Business Media, LLC, pp 91-116
Yoon, Y.J., Kim, D-K., Yoon, C.M., Park, J., Kim, Y-K., Roh, T-Y. and Gho, Y.S (2014) *Egr-1 activation by cancer-derived extracellular vesicles promotes endothelial cell migration via ERK1/2 and JNK signaling pathways* PLoS One, **9**: e115170

Dendritic cells

Kowal, J., Arras, G., Colombo, M., Jouve, M., Morath, J.P., Primdal-Bengtson, B., Dingli, F., Loew, D., Tkach, M. and Théry, C. (2016) *Proteomic comparison defines novel markers to characterize heterogeneous populations of extracellular vesicle subtypes* Proc. Natl. Acad. Sci. USA, **113**, E968–E977
Leone, D.A., Peschel, A., Brown, M., Schachner, H., Ball, M.J., Gyuraszova, M., Salzer-Muhar, U., Fukuda, M. et al (2017) *Surface LAMP-2 is an endocytic receptor that diverts antigen internalized by human dendritic cells into highly immunogenic exosomes* J. Immunol., **199**, 531–546
Mfunyi, C.M., Vaillancourt, M., Vitry, J., Nsimba Batomene, T-R., Posvandzic, A., Lambert, A.A. and Gilbert, C. (2015) *Exosome release following activation of the dendritic cell immunoreceptor: A potential role in HIV-1 pathogenesis* Virology **484**, 103–112
Stremersch, S., Vandenbroucke, R.E., VanWonterghemb, E., Hendrix, A., De Smedt, S.C. and Raemdonck, K. (2016) *Comparing exosome-like vesicles with liposomes for the functional cellular delivery of small RNAs* J. Control. Release, **232**, 51–61

Diabetes mellitus (gestational)

Iljas, J.D., Guanzon, D., Elfeky, O., Rice, G.E. and Salomon, C. (2017) *Bio-compartmentalization of microRNAs in exosomes during gestational diabetes mellitus* Placenta, **54**, 76-82

Drug delivery

Jang, S.C., Kim, O.Y., Yoon, C.M., Choi, D-S., Roh, T-Y., Park, J., Nilsson, J., Lotvall, J., Kim, Y-K. and Gho, Y.S. (2013) *Bioinspired exosome-mimetic nanovesicles for targeted delivery of chemotherapeutics to malignant tumors* ACS Nano, **7**, 7698–7710

Lai, R.C., Yeo, R.W.Y., Tan, K.H. and Lim, S.K. (2013) *Exosomes for drug delivery — a novel application for the mesenchymal stem cell* Biotechnol. Adv., **31**, 543–551

Vader, P., Mol, E.A., Pasterkamp, G. and Schiffelers, R.M. (2016) *Extracellular vesicles for drug delivery* Adv. Drug Delivery Rev., **106**, 148–156

Embryonic stem cells – see also "Stem cells"

Jo, W., Jeong, D., Kim, J. and Park, J, (2016) *Self-renewal of bone marrow stem cells by nanovesicles engineered from embryonic stem cells* Adv. Healthcare Mater., **5**, 3148–3156

Kim, J., Han, C., Jo, W., Kang, S., Cho, S., Jeong, D., Gho, Y.S. and Park, J. (2017) *Cell-engineered nanovesicle as a surrogate inducer of contact-dependent stimuli* Adv. Healthcare Mater., **6**: 1700381

Endometrial stromal cells

Koh, Y.Q., Peiris, H.N., Vaswani, K., Reed, S., Rice, G.E., Salomon, C. and Mitchell, M.D. (2016) *Characterization of exosomal release in bovine endometrial intercaruncular stromal cells* Reprod. Biol. Endocrinol., **14**: 78

Endothelial cells

Ju, R., Zhuang, Z.W., Zhang, J., Lanahan, A.A., Kyriakides, T., Sessa, W.C. and Simons, M. (2014) *Angiopoietin-2 secretion by endotheliale cell exosomes: regulation by the phosphatidylinositol 3-kinase (PI3K)/Akt/endothelial nitric oxide synthase (eNOS) and syndecan-4/syntenin pathways* J. Biol. Chem.,**289,** 5 10-519

Epididymosomes

Reilly, J.N., McLaughlin, E.A., Stanger, S.J., Anderson, A.L., Hutcheon, K., Church, K., Mihalas, B.P. et al (2016) *Characterisation of mouse epididymosomes reveals a complex profile of microRNAs and a potential mechanism for modification of the sperm epigenome* Sci. Rep., **6**: 31794

Epithelial-mesenchymal transition

Tauro, B.J., Mathias, R.A., Greening, D.W., Gopal, S.K., Ji, H., Kapp, E.A., Coleman, B.M., Hill, A.F., Kusebauch, U. et al (2013) *Oncogenic H-Ras reprograms Madin-Darby Canine Kidney (MDCK) cell-derived exosomal proteins following epithelial-mesenchymal tansition* Mol. Cell. Proteom., **12**: 2148–2159

Epstein Barr virus LMP1

Hurwitz, S.N., Nkosi, D., Conlon, M.M., York, S.B., Liu, X., Tremblay, D.C. and Meckes, D.G. (2017) *CD63 regulates Epstein-Barr virus LMP1 exosomal packaging, enhancement of vesicle production, and noncanonical NF-κB signaling* J. Virol., **91**: e02251-16

Erythrocytes

Equine anaemia

Rout, E.D., Webb, T.L., Laurence, H.M., Long, L. and Olver, C.S. (2015) *Transferrin receptor expression in serum exosomes as a marker of regenerative anaemia in the horse* Equine Vet. J., **47**, 101–106

Plasmodium-infected

Sisquella, X., Ofir-Birin, Y., Pimentel, M.A., Cheng, L., Karam, P.A. Sampaio, N.G., Penington, J.S., Connolly, D. et al (2017) *Malaria parasite DNA-harbouring vesicles activate cytosolic immune sensors* Nat. Comm., **8**: 1985

Regev-Rudzki, N., Wilson, D.W., Carvalho, T.G., Sisquella, X., Coleman, B.M., Rug, M., Bursac, D., Angrisano, F., Gee, M., Hill, A.F., Baum, J. and Cowman, A.F. (2013) *Cell-cell communication between malaria-infected red blood cells via exosome-like vesicles* Cell, **153**, 1120–1133

Exosome profiling

Akagi, T. and Ichiki, T. (2017) *Microcapillary chip-based extracellular vesicle profiling system* In Extracellular Vesicles: Methods and Protocols, Methods Mol. Biol., **1660**, (ed. Kuo, W.P. and Jia, S.) Springer Science+Business Media, LLC, pp 209-217

Exosome size

Minciacchi, V.R., Spinelli, C., Reis-Sobreiro, M., Cavallini, L., You, S., Zandian, M., Li, X., Mishra, R., Chiarugi, P., Adam, R.M. et al (2017) *MYC mediates large oncosome-induced fibroblast reprogramming in prostate cancer* Cancer Res., **77**, 2306-2317

Exosomes *versus* microvesicles

Collino, F., Pomatto, M., Bruno, S., Lindoso, R.S., Tapparo, M., Sicheng, W., Quesenberry, P. and Camussi, G. (2017) *Exosome and microvesicle-enriched fractions isolated from mesenchymal stem cells by gradient separation showed different molecular signatures and functions on renal tubular epithelial cells* Stem Cell Rev. Rep., **13**, 226–243

Eye diseases

Klingeborn, M., Dismuke, W.M., Rickman, C.B. and Stamer, W.D. (2017) *Roles of exosomes in the normal and diseased eye* Prog. Retin. Eye Res., **59**, 158-177

Fibrosarcoma cells

Sung, B.H., Ketova, T., Hoshino, D., Zijlstra, A. and Weaver, A.M. (2015) *Directional cell movement through tissues is controlled by exosome secretion* Nat. Commun., **6**: 7164

Gatrointestinal cancers

Lindner, K., Haier, J., Wang, Z., Watson, D.I., Hussey, D.J. and Hummel, R. (2015) *Circulating microRNAs: emerging biomarkers for diagnosis and prognosis in patients with gastrointestinal cancers* Clin. Sci., **128**, 1–15

Gene delivery

Lamichhane, T.N., Raiker, R.S. and Jay, S.M. (2015) *Exogenous DNA loading into extracellular vesicles via electroporation is size-dependent and enables limited gene delivery* Mol. Pharmaceut., **12**, 3650−3657

Glioblastoma

Choi, D-S., Montermini, L. and Rak, J. (2017) *The impact of oncogenic EGFRvIII on the proteome of extracellular vesicles released from glioblastoma cells* J. Extracell. Vesicles Abstr. PT07.10

De Vrij, J., Maas, S.L.N., Kwappenberg, K.M.C., Schnoor, R., Kleijn, A., Dekker, L., Luider, T.M., de Witte, L.D., Litjens, M. et al (2015) *Glioblastoma-derived extracellular vesicles modify the phenotype of monocytic cells* Int. J. Cancer, **137**, 1630–1642

Mallawaaratchy, D.M., Hallal, S., Russell, B., Ly, L., Ebrahimkhani, S., Wei, H., Christopherson, R.I., Buckland, M.E. and Kaufman, K.L. (2017) *Comprehensive proteome profiling of glioblastoma-derived extracellular vesicles identifies markers for more aggressive disease* J. Neurooncol., **131**, 233–244

Glomerular mesenchymal stromal

Ranghino, A., Bruno, S., Bussolati, B., Moggio, A., Dimuccio, V., Tapparo, M., Biancone, L., Gontero, P., Frea, B. and Camussi, G. (2017) *The effects of glomerular and tubular renal progenitors and derived extracellular vesicles on recovery from acute kidney injury* Stem Cell Res. Ther., **8** :24

Head and neck cancers

Principe, S., Hui, A.B-Y., Bruce, J., Sinha, A., Liu, F-F. and Kislinger, T. (2013) T*umor-derived exosomes and microvesicles in head and neck cancer: Implications for tumor biology and biomarker discovery* Proteomics **13**, 1608–1623

HEK cells

Cabezas, S.C. and Federico, M. (2013) *Sequences within RNA coding for HIV-1 Gag p17 are efficiently targeted to exosomes* Cell. Microbiol., **15**, 412–429

El-Andaloussi, S., Lee, Y., Lakhal-Littleton, S., Li, J., Seow, Y., Gardiner, C., Alvarez-Erviti, L., Sargent, I.L. and Wood, M.J.A. (2012) *Exosome-mediated delivery of siRNA in vitro and in vivo* Nat. Protocols, **7**, 2112-2126

Hurwitz, S.N., Conlon, M.M., Rider, M.A., Brownstein, N.C. and Meckes, Jr, D.G. (2016) *Nanoparticle analysis sheds budding insights into genetic drivers of extracellular vesicle biogenesis* J. Extracell. Vesicles, **5**: 31295

Hurwitz, S.N., Nkosi, D., Conlon, M.M., York, S.B., Liu, X., Tremblay, D.C. and Meckes, D.G. (2017) *CD63 regulates Epstein-Barr virus LMP1 exosomal packaging, enhancement of vesicle production, and noncanonical NF-κB signaling* J. Virol., **91**: e02251-16
Kong, S.M.Y., Chan, B.K.K., Park, J-S., Hill, K.J., Aitken, J.B., Cottle, L., Farghaian, H., Cole, A.R., Lay, P.A., Sue, C.M. and Cooper, A.A. (2014) *Parkinson's disease-linked human PARK9/ATP13A2 maintains zinc homeostasis and promotes α-Synuclein externalization via exosomes* Hum. Mol. Genet., **23**, 2816–2833
Kooijmans, S.A.A., Stremersch, S., Braeckmans, K., de Smedt, S.C., Hendrix, A., Wood, M.J.A., Schiffelers, R.M., Raemdonck, K. and Vader, P. (2013) *Electroporation-induced siRNA precipitation obscures the efficiency of siRNA loading into extracellular vesicles* J. Control. Release, **172**, 229-238
Lamichhane, T.N., Raiker, R.S. and Jay, S.M. (2015) *Exogenous DNA loading into extracellular vesicles via electroporation is size-dependent and enables limited gene deleivery* Mol. Pharmaceut., **12**, 3650-3657
Luo, X., Fan, Y., Park, I-W. and He, J.J. (2015) *Exosomes are unlikely involved in intercellular Nef transfer* PLoS One, **10**: e0124436
Majumdar, R., Tavakoli Tameh, A. and Parent, C.A. (2016) *Exosomes mediate LTB4 release during neutrophil chemotaxis* PLoS Biol., **14**: e1002336
Mangeot, P-E., Dollet, S., Girard, M., Ciancia, C., Joly, S., Peschanski, M. and Lotteau, V. (2011) *Protein transfer into human cells by VSV-G-induced nanovesicles* Mol. Ther., **19**, 1656–1666
Mukherjee, K., Ghoshal, B., Ghosh, S., Chakrabarty, Y., Shwetha, S., Das, S. and Bhattacharyya, S.N. (2016) *Reversible HuR-microRNA binding controls extracellular export of miR-122 and augments stress response* EMBO Rep., **17**, 1184-1203
Putz, U., Mah, S., Goh, C-P., Low, L-H., Howitt, J. and Tan, S-S. (2015) *PTEN secretion in exosomes* Methods, **77–78**, 57–163
Ruiss, R., Jochum, S., Mocikat, R., Hammerschmidt, W. and Zeidler, R. (2011) *EBV-gp350 confers B-cell tropism to tailored exosomes and is a neo-antigen in normal and malignant B cells - new option for the treatment of B-CLL* PLoS One, **6**: e25294

Hepatitis C virus particles/heptoma cells

Liu, Z., Zhang, X., Yu, Q. and He, J.J. (2014) *Exosome-associated hepatitis C virus in cell cultures and patient plasma* Biochem. Biophys. Res. Comm., **455**, 218–222
Ren, H., Elgner, F., Himmelsbach, K., Akhras, S., Jiang, B., Medvedev, R., Ploen, D. and Hildt, E. (2017) *Identification of syntaxin 4 as an essential factor for the hepatitis C virus life cycle* Eur. J. Cell Biol., **96**, 542–552

Hepatocytes

Thacker, S., Nautiyal, M., Holman, N., Otieno, M., Watkins, P. and Mosedale, M. (2017) *Hepatocyte-derived exosome enrichment and cell culture methods optimisation for the identification of novel DILI biomarkers* J. Extracell. Vesicles Abstr. PT06.06

Herpes simplex virus infection

Kalamvoki, M. and Deschamps, T. (2016) *Extracellular vesicles during Herpes Simplex Virus type 1 infection: an inquiry* Virol. J., **13**: 63

HIV infected cells

Cabezas, S.C. and Federico, M. (2013) *Sequences within RNA coding for HIV-1 Gag p17 are efficiently targeted to exosomes* Cell. Microbiol., **15**, 412–429
Hu, G., Yelamanchili, S., Kashanchi, F., Haughey, N., Bond, V.C., Witwer, K.W., Pulliam, L. and Buch, S. (2017) *Proceedings of the 2017 ISEV symposium on "BHIV, NeuroHIV, drug abuse, & EVs"* J. Neurovirol. **23**: 935–940
Rahimian, P. and He, J.J. (2016) *Exosome-associated release, uptake, and neurotoxicity of HIV-1 Tat protein* J. Neurovirol., **22**, 774–788

HTLV infected cells

Barclay, R.A., Pleet, M.L., Akpamagbo, Y., Noor, K., Mathiesen, A. and Kashanchi, F. (2017) *Isolation of exosomes from HTLV-infected cells* In Human T-Lymphotropic Viruses, Methods and Protocols: Methods Mol. Biol., **1582** (ed. Hill, A.F.), Springer Science+Business Media, LLC, pp 57-75

Human CD4$^+$ cells

Rahimian, P. and He, J.J. (2016) *Exosome-associated release, uptake, and neurotoxicity of HIV-1 Tat protein* J. Neurovirol., **22**, 774–788

Human breast carcinoma

Ettelaie, C., Collier, M.E.W., Maraveyas, A. and Ettelaie, R. (2014) *Characterization of physical properties of tissue factor-containing microvesicles and a comparison of ultracentrifuge-based recovery procedures* J. Extracell. Vesicles **3**: 23592

Van Deun, J., Mestdagh, P., Sormunen, R., Cocquyt, V., Vermaelen, K., Vandesompele, J., Bracke, M., De Wever, O. and Hendrix, A. (2014) *The impact of disparate isolation methods for extracellular vesicles on downstream RNA profiling* J. Extracell. Vesicles **3**: 24858

Human hepatic cells

Mukherjee, K., Ghoshal, B., Ghosh, S., Chakrabarty, Y., Shwetha, S., Das, S. and Bhattacharyya, S.N. (2016) *Reversible HuR-microRNA binding controls extracellular export of miR-122 and augments stress response* EMBO Rep., **17**, 1184-1203

Human kidney epithelial

Rahimian, P. and He, J.J. (2016) *Exosome-associated release, uptake, and neurotoxicity of HIV-1 Tat protein* J. Neurovirol., **22**, 774–788

Human liver stem cells

Herrera Sanchez, M.B., Previdi, S., Bruno, S., Fonsato, V., Deregibus, M.C., Kholia, S., Petrillo, S., Tolosano, E., Critelli, R. et al (2017) *Extracellular vesicles from human liver stem cells restore argininosuccinate synthase deficiency* Stem Cell Res. Ther., **8**: 176

Human lung carcinoma cells

Clark, D.J., Fondrie, W.E., Yang, A. and Mao, L. (2016) *Triple SILAC quantitative proteomic analysis reveals differential abundance of cell signaling proteins between normal and lung cancer-derived exosomes* J. Proteom., **133**, 161–169

Human myelomonocytic cells

Zargarian, S., Shlomovitz, I., Erlich, Z., Hourizadeh, A., Ofir-Birin, Y., Croker, B., Regev-Rudzki, N., Edry-Botzer, L. and Gerlic, M. (2017) *Phosphatidylserine externalization, "necroptotic bodies" release, and phagocytosis during necroptosis* PLoS Biol. **15**: e2002711

Human saliva

Iwai, K., Minamisawa, T., Suga, K., Yajima, Y. and Shiba, K. (2016) *Isolation of human salivary extracellular vesicles by iodixanol density gradient ultracentrifugation and their characterization* J. Extracell. Vesicles, **5**: 30829

Human sperm (acrosome)

Zhou, W., Anderson, A.L., Turner, A.P., De Iuliis, G.N., McCluskey, A., McLaughlin, E.A. and Nixon, B. (2017) *Characterization of a novel role for the dynamin mechanoenzymes in the regulation of human sperm acrosomal exocytosis* Mol. Hum. Reprod., **23**, 657–673

HUVEC-derived

Salomon, C., Scholz-Romero, K., Sarker, S., Sweeney, E., Kobayashi, M., Correa, P., Longo, S.Duncombe, G., Mitchell, M.D., Rice, G.E. and Illanes, S.E. (2016) *Gestational diabetes mellitus is associated with changes in the concentration and bioactivity of placenta-derived exosomes in maternal circulation across gestation* Diabetes, **65,** 598–609

Hypothalamus

Soetedjo, L. and Jin, H. (2014) *Agonist-induced GPCR shedding from the ciliary surface is dependent on ESCRT-III and VPS4* Curr., Biol., **24**, 509-518

Immunogenicity

Leone, D.A., Peschel, A., Brown, M., Schachner, H., Ball, M.J., Gyuraszova, M., Salzer-Muhar, U., Fukuda, M. et al (2017) *Surface LAMP-2 is an endocytic receptor that diverts antigen internalized by human dendritic cells into highly immunogenic exosomes* J. Immunol., **199**, 531–546

Intestinal epithelial cells

Hasegawa, H., Thomas, H.J., Schooley, K. and Born, T.L. (2011) *Native IL-32 is released from intestinal epithelial cells via a non-classical secretory pathway as a membrane-associated protein* Cytokine, **53**, 74–83

Jurkat cells

Martin-Jaular, L., Liao, Z., Gerber, P.P., Ostrowski, M., Witwer, K. and Théry, C. (2017) *Attempts to re-define cellular components specifically incorporated in HIV as compared to sEVs and exosomes secreted by infected cells* J. Extracell. Vesicles Abstr. OF18.02

Németh, A., Orgovan, N., Sódar, B.W., Osteikoetxea, X., Pálóczi, K., Szabó-Taylor, K.E., Vukman, K.V., Kittel, A. et al (2017) *Antibiotic-induced release of small extracellular vesicles (exosomes) with surface-associated DNA* Sci. Rep., **7**: 8202

Keratinocytes

Lo Cicero, A., Delevoye, C., Gilles-Marsens, F., Loew, D., Dingli F., Guéré, André, N., Vié, K., van Niel, G. and Raposo, G. (2015) *Exosomes released by keratinocytes modulate melanocyte pigmentation* Nature Comm., **6**:7506

Kidney cells

Wang, X., Wilkinson, R., Kildey, K., Potriquet, J., Mulvenna, J., Lobb, R.J., Möller, A., Cloonan, N. et al (2017) *Unique molecular profile of exosomes derived from primary human proximal tubular epithelial cells under diseased conditions* J. Extracell. Ves., **6**: 1314073

Zhang, W., Zhou, X., Zhang, H., Yao, Q., Liu, Y., Dong, Z. (2016) *Extracellular vesicles in diagnosis and therapy of kidney diseases* Am. J. Physiol. Renal Physiol., **311**, F844–F851

Liver fibrosis

Chen, L. and Brigstock, D.R. (2017) *Cellular or exosomal microRNAs associated with CCN gene expression in liver fibrosis* In CCN Proteins: Methods and Protocols, Methods Mol. Biol., **1489** (ed. Takigawa, M.) Springer Science+Business Media, New York, pp 465-480

Lung cancer cells

Choi, D-Y., You, S., Jung, J.H., Lee, J.C., Rho, J.K., Lee, K.Y., Freeman, M.R., Kim, K.P. and Kim, J. (2014) *Extracellular vesicles shed from gefitinib-resistant nonsmall cell lung cancer regulate the tumor microenvironment* Proteomics 2014, 14, 1845–1856

Clark, D.J., Fondrie, W.E., Yang, A. and Mao, L. (2016) *Triple SILAC quantitative proteomic analysis reveals differential abundance of cell signaling proteins between normal and lung cancer-derived exosomes* J. Proteom., **133**, 161–169

Jung, J.H., Lee, M.Y., Choi, D-Y., Lee, J.W., You, S., Lee, K.Y., Kim, J. and Kim, K.P. (2015) *Phospholipids of tumor extracellular vesicles stratify gefitinib-resistant nonsmall cell lung cancer cells from gefitinib-sensitive cells* Proteomics, **15**, 824–835

Lia, J., Yuc, J., Liu, A. and Wang, Y. (2014) *β-Elemene against human lung cancer via up-regulation of P53protein expression to promote the release of exosome* Lung Cancer, **86,** 144–150

Lobb, R.J., Becker, M., Wen, S.W., Wong, C.S.F., Wiegmans, A.P., Leimgruber, A. and Andreas Möller C. (2015) *Optimized exosome isolation protocol for cell culture supernatant and human plasma* J. Extracell. Vesicles **4**: 27031

Park, J.O., Choi, D-Y., Choi, D-S., Kim, H.J., Kang, J.W., Jung, J.H., Lee, J.H., Kim, J. et al (2013) *Identification and characterization of proteins isolated from microvesicles derived from human lung cancer pleural effusions* Proteomics, **13**, 2125–2134

Lung tissue (murine)

Lässer, C., Suzuki, S., Park, K-S., Shelke, G., Hovhannisyan, L., Crescitelli, R. and Lötvall, J. (2017) *Proteomic analysis of mouse lung tissue-derived vesicles, a comparison of ultracentrifugation and density flotation isolation* J. Extracell. Vesicles Abstr. PT07.08

Lymphocytic/lymphoblastoid/leukaemia cells/Tcells

Arenaccio, C., Chiozzini, C., Columba-Cabezas, S., Manfredi, F., Affabris, E., Baur, A. and Federico, M. (2014) *Exosomes from human immunodeficiency virus type 1 (HIV-1)-infected cells license quiescent CD4⁺T lymphocytes to replicate HIV-1 through a Nef- and ADAM17-dependent mechanism* J. Virol., **88**, 11529–11539

Cantin, R., Diou, J., Belanger, D., Tremblay, A.M. and Gilbert, C. (2008) *Discrimination between exosomes and HIV-1: Purification of both vesicles from cell-free supernatants* J. Immunol. Methods, **338**, 21-30

Jung, S., Kim, J., Lee, D.J., Oh, E.H., Lim, H., Kim, K.P., Choi, N., Kim, T.S. andf Kim, S.K. (2016) *Extensible multiplex real-time PCR of microRNA using microparticles* Sci. Rep., **6:** 22975

Lenassi, M., Cagney, G., Liao, M., Vaupotĭc, T., Bartholomeeusen, K., Cheng, Y., Krogan, N.J., Plemenită, A. and Peterlin, B.M. (2010) *HIV Nef is secreted in exosomes and triggers apoptosis in bystander CD4⁺ T cells* Traffic, **11**, 110–122

Luo, X., Fan, Y., Park, I-W. and He, J.J. (2015) *Exosomes are unlikely involved in intercellular Nef transfer* PLoS One, **10**: e0124436

Nakai, W., Yoshida, T., Diez, D., Miyatake, Y., Nishibu, T., Imawaka, N., Naruse, K., Sadamura, Y. and Hanayama, R. (2016) *A novel affinity-based method for the isolation of highly purified extracellular vesicles* Sci. Rep., **6**: 33935

Narayanan, A., Iordanskiy, S., Das, R., Van Duyne, R., Santos, S., Jaworski, E., Guendel, I., Sampey, G., Dalby, E., et al (2013) *Exosomes derived from HIV-1-infected cells contain trans-activation response element RNA* J. Biol. Chem., **288**, 20014–20033

Padro, C.J., Shawler, T.M., Gormley, M.G. and Sanders, V.M. (2013) *Adrenergic regulation of IgE involves modulation of CD23 and ADAM10 expression on exosomes* J. Immunol., **191**, 5383–5397

Park, I-W. and He, J.J. (2010) *HIV-1 is budded from CD4⁺ T lymphocytes independently of exosomes* Virol. J., **7**: 234

Sampey, G.C., Saifuddin, M., Schwab, A., Barclay, R., Punya, S., Chung, M-C., Hakami, R.M., Zadeh, M. A. et al (2016) *Exosomes from HIV-1-infected cells stimulate production of pro-inflammatory cytokines through trans-activating response (TAR) RNA* (2016) J. Biol. Chem., **291**, 1251–1266

Thompson, C.A., Purushothaman, A., Ramani, V.C., Vlodavsky, I. and Sanderson, R.D. (2013) *Heparanase regulates secretion, composition, and function of tumor cell-derived exosomes* J. Biol. Chem., **288**, 10093–10099

Macrophages (see "Monocytes")

Mast cells

Cvjetkovic, A., Jang, S.C., Konečná, B., Höög, J.L., Sihlbom, C., Lässer, C. and Lötvall, J. (2016) *Detailed analysis of protein topology of extracellular vesicles–evidence of unconventional membrane protein orientation* Sci. Rep., **6**: 36338

Kormelink, T.G., Arkesteijn, G.J.A., van de Lest, C.H.A., Geerts, W.J.C., Goerdayal, S.S., Altelaar, M.A.F., Redegeld, F.A., Nolte-'t Hoen, E.N.M. and Wauben, M.H.M. (2016) *Mast cell degranulation is accompanied by the release of a selective subset of extracellular vesicles that contain mast cell–specific proteases* J. Immunol., **197,** 382–3392

Rupert, D.L.M., Shelke, G.V., Emilsson, G., Claudio, V., Block, S. Lässer, C., Dahlin, A., Lötvall, J.O., Bally, M., Zhdanov, V.P. and Höök, F. (2016) *Dual-wavelength surface plasmon resonance for determining the size and concentration of sub-populations of extracellular vesicles* Anal. Chem., **88**, 9980−9988

Shelke, G. and Lötvall, J. (2017) *Recipient cell organelle separation for EV uptake studies: Tracking of extracellular vesicles* J. Extracell. Vesicles Abstr. LBP.45

Zabeo, D., Cvjetkovic, A., Lässer, C., Schorb, M., Lötvall, J. and Höög, J.L. (2017) *Exosomes purified from a single cell type have diverse morphology* J. Extracell. Ves., **6**: 1329476

MDCK cells

Casas, E., Barron, C., Francis, S.A., McCormack, J.M., McCarthy, K.M., Schneeberger, E.E. and Lynch, R.D. (2010) *Cholesterol efflux stimulates metalloproteinase-mediated cleavage of occludin and release of extracellular membrane particles containing its C-terminal fragments* Exp. Cell Res., **316**, 353-365

Tauro, B.J., Mathias, R.A., Greening, D.W., Gopal, S.K., Ji, H., Kapp, E.A., Coleman, B.M., Hill, A.F. et al (2013) *Oncogenic H-Ras reprograms Madin-Darby Canine Kidney (MDCK) cell-derived exosomal proteins following epithelial-mesenchymal tansition* Mol. Cell. Proteom., **12**: 2148–2159

Medulloblastoma cells

Epple, L.M., Griffiths, S.G., Dechkovskaia, A.M., Dusto, N.L., White, J., Ouellette, R.J., Anchordoquy, T.J., Bemis, L.T. and Graner, M.W. (2012) *Medulloblastoma exosome proteomics yield functional roles for extracellular vesicles* PLoS One, **7**: e42064

Melanocytes and melanoma cells

Cheung, A.S., Koshy, S.T., Stafford, A.G., Bastings, M.M.C. and Mooney, D.J. (2016) *Adjuvant-loaded subcellular vesicles derived from disrupted cancer cells for cancer vaccination* Small, **12**, 2321–2333

Coscia, C., Parolini, I., Sanchez, M., Biffoni, M., Boussadia, Z., Zanetti, C., Fiani, M.L. and Sargiacomo, M. (2016) *Generation, quantification, and tracing of metabolically labeled fluorescent exosomes* In Lentiviral Vectors and Exosomes as Gene and Protein Delivery Tools, Methods Mol. Biol., **1448** (ed. Federico, M.) Springer Science+Business Media New York 2016, pp 217-235

Ettelaie, C., Collier, M.E.W., Maraveyas, A. and Ettelaie, R. (2014) *Characterization of physical properties of tissue factor-containing microvesicles and a comparison of ultracentrifuge-based recovery procedures* J. Extracell. Vesicles **3**: 23592

Stremersch, S., Marro, M., Pinchasik, B-E., Baatsen, P., Hendrix, A., De Smedt, S.C., Loza-Alvarez, P., Skirtach, A.G., Raemdonck, K. and Braeckmans, K. (2016) *Identification of individual exosome-like vesicles by surface enhanced Raman spectroscopy* Small, **12**, 3292–3301

Van Niel, G., Bergam, P., Di Cicco, A., Hurbain, I., Lo Cicero, A., Dingli, F., Palmulli, R., Fort, C. et al (2015) *Apolipoprotein E regulates amyloid formation within endosomes of pigment cells* Cell Rep., **13**, 43–51

Yamashita, T., Takahashi, Y., Nishikawa, M. and Takakura, Y. (2016) *Effect of exosome isolation methods on physicochemical properties of exosomes and clearance of exosomes from the blood circulation* Eur. J. Pharma. Biopharm., **98**, 1–8

Mesenchymal stem cells – see "Stem cells"

Metabolic syndrome

O'Neill, S., Bohl, M., Gregersen, S., Hermansen, K. and O'Driscoll, L. (2016) *Blood-based biomarkers for metabolic syndrome* Trends Endocrinol. Metab., **27**, 363-374

Metastatic cancer cells

Schillaci, O., Fontana, S., Monteleone, F., Taverna, S., Di Bella, M.A., Di Vizio, D. and Alessandro, R. (2017) *Exosomes from metastatic cancer cells transfer amoeboid phenotype to non-metastatic cells and increase endothelial permeability: their emerging role in tumor heterogeneity* Sci. Rep., **7**: 4711

MicroRNAs

Bellingham, S.A., Shambrook, M. and Hill, A.F. (2017) *Quantitative analysis of exosomal miRNA via qPCR and digital PCR* In Exosomes and Microvesicles Methods and Protocols: Methods Mol. Biol., **1545** (ed. Hill, A.F.), Springer Science+Business Media, LLC, pp 55-70

Benmoussa, A., Ly, S., Shan, S.T., Laugier, J., Boilard, E., Gilbert, C. and Provost, P. (2017) *A subset of extracellular vesicles carries the bulk of microRNAs in commercial dairy cow's milk* J. Extracell. Vesicles, **6**: 1401897

Chen, L. and Brigstock, D.R. (2017) *Cellular or exosomal microRNAs associated with CCN gene expression in liver fibrosis* In CCN Proteins: Methods and Protocols, Methods Mol. Biol., **1489** (ed. Takigawa, M.) Springer Science+Business Media, New York, pp 465-480

Conley, A., Minciacchi, V.R., Lee, D.H., Knudsen, B.S., Karlan, B.Y., Citrigno, L., Viglietto, G. et al (2017) *High-throughput sequencing of two populations of extracellular vesicles provides an mRNA signature that can be detected in the circulation of breast cancer patients* RNA Biol., **14**, 305–316

Iljas, J.D., Guanzon, D., Elfeky, O., Rice, G.E. and Salomon, C. (2017) *Bio-compartmentalization of microRNAs in exosomes during gestational diabetes mellitus* Placenta, **54**, 76-82

Lindner, K., Haier, J., Wang, Z., Watson, D.I., Hussey, D.J. and Hummel, R. (2015) *Circulating microRNAs: emerging biomarkers for diagnosis and prognosis in patients with gastrointestinal cancers* Clin. Sci., **128**, 1–15

Quek, C., Bellingham, S.A., Jung, C-H., Scicluna, B.J., Shambrook, M.C., Sharples, R.A., Cheng, L. and Hill, A.F. (2017) *Defining the purity of exosomes required for diagnostic profiling of small RNA suitable for biomarker discovery* RNA Biol., **14**, 245–258

Ying, W., Riopel, M., Bandyopadhyay, G., Dong, Y., Birmingham, A., Seo, J.B., Ofrecio, J.M., Wollam, J. et al (2017) *Adipose tissue macrophage-derived exosomal miRNAs can modulate in vivo and in vitro insulin sensitivity* Cell, 171, 372–384

Milk (bovine)

Abderrahim, B., Sophia, L., Ting, S.S., Laugier, J., Boilard, E., Caroline, G. and Provost, P. (2017) *Characterisation of extracellular vesicles with milk fat globule membrane-like properties that carry most microRNAs in commercial dairy cow milk* J. Extracell. Vesicles Abstr. OS21.02

Benmoussa, A., Lee, C.H.C., Laffont, B., Savard, P., Laugier, J., Boilard, E., Gilbert, C., Fliss, I. and Provost, P. (2016) *Commercial dairy cow milk microRNAs resist digestion under simulated gastrointestinal tract conditions* J. Nutr., **146**, 2206–2215

Benmoussa, A., Ly, S., Shan, S.T., Laugier, J., Boilard, E., Gilbert, C. and Provost, P. (2017) *A subset of extracellular vesicles carries the bulk of microRNAs in commercial dairy cow's milk* J. Extracell. Vesicles, **6**: 1401897

Munagala, R., Aqil, F., Jeyabalan, J. and Gupta, R.C. (2016) *Bovine milk-derived exosomes for drug delivery* Cancer Lett., **371**, 48–61

Samuel, M., Chisanga, D., Liem, M., Keerthikumar, S., Anand, S., Ang, C-S., Adda, C.G., Versteegen, E., Jois, M. and Mathivanan, S. (2017) *Bovine milk-derived exosomes from colostrum are enriched with proteins implicated in immune response and growth* Sci. Rep., **7**: 5933

Samuel, M., Jois, M. and Mathivanan, S. (2017) *Exosomes from bovine milk reduce the tumour burden and attenuates cancer cachexia* J. Extracell. Vesicles Abstr. OT9.02

Monocytes/macrophages

Deshmane, S., Sheffield, J., Khalili, K. and Datta, P. (2015) *Characterization of extracellular vesicles (exosomes) from HIV-1 infected macrophages treated with HIV-1 protease inhibitor, Ritonavir* J. Neurovirol., **21 (Suppl. 1)** S17

Hwang, D.W., Choi, H., Jang, S.C., Yoo, M.Y., Park, J.Y., Choi, N.E., Oh, H.J., Ha, S. et al (2015) *Noninvasive imaging of radiolabeled exosome-mimetic nanovesicle using ^{99m}Tc-HMPAO* Sci. Rep., **5**: 15636

Nair, R.R., Mazza, D., Agresti, A. and Bianchi, M. (2017) *Histone flow: from nucleus to extracellular vesicles* J. Extracell. Vesicles Abstr. OS24.04

Németh, A., Orgovan, N., Sódar, B.W., Osteikoetxea, X., Pálóczi, K., Szabó-Taylor, K.E., Vukman, K.V., Kittel, A. et al (2017) *Antibiotic-induced release of small extracellular vesicles (exosomes) with surface-associated DNA* Sci. Rep., **7**: 8202

Osada-Oka, M., Shiota, M., Izumi, Y., Nishiyama, M., Tanaka, M., Yamaguchi, T., Sakurai, E., Miura, K. and Iwao, H. (2017) *Macrophage-derived exosomes induce inflammatory factors in endothelial cells under hypertensive conditions* Hypertens. Res., **40**, 353–360

Ying, W., Riopel, M., Bandyopadhyay, G., Dong, Y., Birmingham, A., Seo, J.B., Ofrecio, J.M., Wollam, J. et al (2017) *Adipose tissue macrophage-derived exosomal miRNAs can modulate in vivo and in vitro insulin sensitivity* Cell, 171, 372–384

Zhang, Y., Liu, D., Chen, X., Li, J., Li, L., Bian, Z., Sun, F., Lu, J., Yin, Y., Cai, X., et al (2010) *Secreted monocytic miR-150 enhances targeted endothelial cell migration* Mol. Cell, **39**, 133–144

Mouse embryo fibroblasts

Lunavat, T.R., Jang, S.C., Nilsson, L., Park, H.T., Repiska, G., Lässer, C., Nilsson, J.A., Gho, Y.S. and Lötvall, J. (2016) *RNAi delivery by exosome-mimetic nanovesicles - Implications for targeting c-Myc in cancer* Biomaterials, **10**, 231-238

Myeloma cells

Purushothaman, A., Bandari, S.K., Liu, J., Mobley, J.A., Brown, E.E. and Sanderson, R.D. (2016) *Fibronectin on the surface of myeloma cell-derived exosomes mediates exosome-cell interactions* J. Biol. Chem., **291**, 1652–1663

Thompson, C.A., Purushothaman, A., Ramani, V.C., Vlodavsky, I. and Sanderson, R.D. (2013) *Heparanase regulates secretion, composition, and function of tumor cell-derived exosomes* J. Biol. Chem., **288**, 10093–10099

Multiple myeloma serum

Paolini, L., Zendrini, A., Di Noto, G., Busatto, S., Lottini, E., Radeghieri, A., Dossi, A., Caneschi, A., Ricotta, D. and Bergese, P. (2016) *Residual matrix from different separation techniques impacts exosome biological activity* Sci. Rep., **6**: 23550

Necroptosis

Zargarian, S., Shlomovitz, I., Erlich, Z., Hourizadeh, A., Ofir-Birin, Y., Croker, B., Regev-Rudzki, N., Edry-Botzer, L. and Gerlic, M. (2017) *Phosphatidylserine externalization, "necroptotic bodies" release, and phagocytosis during necroptosis* PLoS Biol. **15**: e2002711

NK cells

Jong, A.Y., Wu, C-H., Li, J., Sun, J., Fabbri, M., Wayne, A.S. and Seeger, R.C. (2017) *Large-scale isolation and cytotoxicity of extracellular vesicles derived from activated human natural killer cells* J. Extracell. Vesicles, **6**: 1294368

Neuroblastoma cells

Nakata, R., Shimada, H., Fernandez, G.E., Fanter, R., Fabbri, M., Malvar, J. Zimmermann, P. and DeClerck, Y.A. (2017) *Contribution of neuroblastoma-derived exosomes to the production of pro-tumorigenic signals by bone marrow mesenchymal stromal cells* J. Extracell. Ves., **6**: 1332941

Nakata, R., Sarte, L., Zimmermann, P. and DeClerck, Y.A. (2017) *Galectin-3 binding protein present at the surface of tumour exosomes contributes to their capture by stromal cells* J. Extracell. Vesicles Abstr. PF04.13

Neural cells

De Rivero Vaccari, J.P., Brand III, F., Adamczak, S., Lee, S.W., Perez-Barcena, J., Wang, M.Y., Bullock, M.R., Dietrich, W.D. and Keane, R.W. (2016) *Exosome-mediated inflammasome signaling aftercentral nervous system injury* J. Neurochem., **136 (Suppl. 1)**, 39-48

Hwang, D.W., Choi, H., Jang, S.C., Yoo, M.Y., Park, J.Y., Choi, N.E., Oh, H.J., Ha, S. et al (2015) *Noninvasive imaging of radiolabeled exosome-mimetic nanovesicle using ^{99m}Tc-HMPAO* Sci. Rep., **5**: 15636

Kanninen, K.M., Bister, N., Koistinaho, J. and Malm, T. (2016) *Exosomes as new diagnostic tools in CNS diseases* Biochim. Biophys. Acta, **1862**, 403–410

Quek, C., Jung, C-h., Bellingham, S.A., Lonie, A. and Hill, A.F. (2015) *ISRAP - a one-touch research tool for rapid profiling of small RNA-seq data* J. Extracell. Vesicles **4**: 29454

Sampey, G.C., Meyering, S.S., Zadeh, M.A., Saifuddin, M.,, Hakami, R.M. and Kashanchi, F. (2014) *Exosomes and their role in CNS viral infections* J. Neurovirol., **20**, 199−208

Neutrophils

Majumdar, R., Tavakoli Tameh, A. and Parent, C.A. (2016) *Exosomes mediate LTB4 release during neutrophil chemotaxis* PLoS Biol., **14**: e1002336

Oncosomes – see Prostate cancer cells

Pancreatic β-cells

Bosch, S., de Beaurepaire, L., Allard, M., Mosser, M., Heichette, C., Chrétien, D., Jegou, D. and Bach, J-M. (2016) *Trehalose prevents aggregation of exosomes and cryodamage* Sci. Rep., **6**: 36162

Pancreatic carcinoma cells

Ettelaie, C., Collier, M.E.W., Maraveyas, A. and Ettelaie, R. (2014) *Characterization of physical properties of tissue factor-containing microvesicles and a comparison of ultracentrifuge-based recovery procedures* J. Extracell. Vesicles **3**: 23592

Kamerkar, S., LeBleu, V.S., Sugimoto, H., Yang, S., Ruivo, C.F., Melo, S.A., Lee, J.J. and Kalluri, R. (2017) *Exosomes facilitate therapeutic targeting of oncogenic KRAS in pancreatic cancer* Nature 546, 498-503

Klein-Scory, S., Tehrani, M.M., Eilert-Micus, C., Adamczyk, K.A., Wojtalewicz, N. Schnölzer, M.. Hahn, S.A., Schmiegel, W. and Schwarte-Waldhoff, I. (2014) *New insights in the composition of extracellular vesicles from pancreatic cancer cells: implications for biomarkers and functions* Proteome Sci., **12**: 50

Mertens, I., Castiglia, M., Carreca, A.P., Baggertman, G., Peeters, M., Pauwels, P. and Rolfo, C. (2014) *Exosome analysis in cancer patients: From the preclinical towards the clinical application: Trial design* Eur. J. Cancer, **50**, Suppl. 6, 96

Németh, A., Orgovan, N., Sódar, B.W., Osteikoetxea, X., Pálóczi, K., Szabó-Taylor, K.E., Vukman, K.V., Kittel, A. et al (2017) *Antibiotic-induced release of small extracellular vesicles (exosomes) with surface-associated DNA* Sci. Rep., **7**: 8202

Papillomavirus

Petrik, J. (2016) *Immunomodulatory effects of exosomes produced by virus-infected cells* Transfus. Apher. Sci., **55**, 84–91

Plasma

Bovine

Crookenden, M.A., Dukkipati, V.S.R., Kay, J.K., Meier, S., Roche, J.R. and Mitchell, M.D. (2016) *Proteins from circulating exosomes represent metabolic state in transition dairy cows* J. Dairy Sci., **99**, 7661–7668

Mitchell, M.D., Scholz-Romero, K., Reed, S., Peiris, H.N., Koh, Y.Q., Meier, S., Walker, C.G., Burke, C.R., Roche, J.R., Rice, G. and Salomon, C. (2016) *Plasma exosome profiles from dairy cows with divergent fertility phenotypes* J. Dairy Sci. **99**, 7590–7601

Human

Bæk, R., Søndergaard, E.K.L., Varming, K. and Jørgensen, M.M. (2016) *The impact of various preanalytical treatments on the phenotype of small extracellular vesicles in blood analyzed by protein microarray* J. Immunol. Meth., **438**, 11–20

Cavallari, C., Ranghino, A., Tapparo, M., Cedrino, M., Figliolini, F., Grange, C., Giannachi, V., Garneri, P. et al (2017) *Serum-derived extracellular vesicles (EVs) impact on vascular remodeling and prevent muscle damage in acute hind limb ischemia* Sci. Rep., **7**: 8180

Elfeky, O., Longo, S., Lai, A., Rice, G.E. and Salomon, C. (2017) *Influence of maternal BMI on the exosomal profile during gestation and their role on maternal systemic inflammation* Placenta, **50**, 60-69

Fernando, M.R., Jiang, C., Krzyzanowski, G.D. and Ryan, W.L. (2017) *New evidence that a large proportion of human blood plasma cell-free DNA is localized in exosomes* PLoS One, **12**: e0183915

Guescini, M., Canonico, B., Lucertini, F., Maggio, S., Annibalini, G., Barbieri, E., Luchetti, F., Papa, S. and Stocchi, V. (2015) *Muscle releases alpha-sarcoglycan positive extracellular vesicles carrying miRNAs in the bloodstream* PLoS One, **10**: e0125094

Kalra, H., Adda, C.G., Liem, M., Ang, C-S., Mechler, A., Simpson, R.J., Hulett, M.D. and Mathivanan, S. (2013) *Comparative proteomics evaluation of plasma exosome isolation techniques and assessment of the stability of exosomes in normal human blood plasma* Proteomics, **13**, 3354–3364

Konadu, K.A., Chu, J., Huang, M.B., Amancha, P.K., Armstrong, W., Powell, M.D., Villinger, F. and Bond, V.C. (2015) *Association of cytokines with exosomes in the plasma of HIV-1–seropositive individuals* J. Infect. Dis., **211**, 1712–1716

Lobb, R.J., Becker, M., Wen, S.W., Wong, C.S.F., Wiegmans, A.P., Leimgruber, A. and Andreas Möller C. (2015) *Optimized exosome isolation protocol for cell culture supernatant and human plasma* J. Extracell. Vesicles **4**: 27031

Meningher, T., Lerman, G., Regev-Rudzki, N., Gold, D., Ben-Dov, I.Z., Sidi, Y., Avni, D. and Schwartz, E. (2017) *Schistosomal microRNAs isolated from extracellular vesicles in sera of infected patients: a new tool for diagnosis and follow-up of human schistosomiasis* J. Infect. Dis., **215**, 378–86

Minciacchi, V.R., Spinelli, C., Reis-Sobreiro, M., Cavallini, L., You, S., Zandian, M., Li, X., Mishra, R., Chiarugi, P., Adam, R.M. et al (2017) *MYC mediates large oncosome-induced fibroblast reprogramming in prostate cancer* Cancer Res., **77**, 2306-2317

Morgan, R.L., Behbahani-Nejad, N., Endres, J., Amin, M.A., Lepore, N.J., Du, Y., Urquhart, A., Chung, K.C. and Fox, D.A. (2016) *Localization, shedding, regulation and function of aminopeptidase N/CD13 on fibroblast like synoviocytes* PloS One, **11**: e0162008

Ouyang, Y., Bayer, A., Chu, T., Tyurin, V., Kagan, V., Morelli, A.E., Coyne, C.B. and Sadovsky, Y. (2016) *Isolation of human trophoblastic extracellular vesicles and characterization of their cargo and antiviral activity* Placenta, **47**, 86-95

Prieto, D., Sotelo, N., Seija, N., Sernbo, S., Abreu, C., Durán, R., Gil, M., Sicco, E. et al (2017) *S100-A9 protein in exosomes from chronic lymphocytic leukemia cells promotes NF-κB activity during disease progression* Blood, **130**, 777-788

Salomon, C., Scholz-Romero, K., Sarker, S., Sweeney, E., Kobayashi, M., Correa, P., Longo, S.Duncombe, G., Mitchell, M.D., Rice, G.E. and Illanes, S.E. (2016) *Gestational diabetes mellitus is associated with changes in the concentration and bioactivity of placenta-derived exosomes in maternal circulation across gestation* Diabetes, **65,** 598–609

Salomon, C., Guanzon, D., Scholz-Romero, K., Longo, S., Correa, P., Illanes, S.E. and Rice, G.E. (2017) *Placental exosomes as early biomarker of preeclampsia: potential role of exosomal microRNAs across gestation* J. Clin. Endocrinol. Metab., **102**, 3182–3194

Shi, M., Liu, C., Cook, T.J., Bullock, K.M. et al (2014) *Plasma exosomal α-synuclein is likely CNS-derived and increased in Parkinson's disease* Acta Neuropathol., **128**, 639–650

Sódar, B.W., Kittel, A., Pálóczi, K., Vukman, K.V., Osteikoetxea, X., Szabó-Taylor, K., Németh, A., Sperlágh, B., Baranyai, T. et al (2016) *Low-density lipoprotein mimics blood plasma-derived exosomes and microvesicles during isolation and detection* Sci. Rep., **6**: 24316

Thind, A. and Wilson, C. (2016) *Exosomal miRNAs as cancer biomarkers and therapeutic targets* J. Extracell. Vesicles, **5**: 31292

Wua, M., Ouyang, Y., Wang, Z., Zhang, R., Huang, P-H., Chen, C., Li, H., Li, P., Quinn, D. et al (2017) *Isolation of exosomes from whole blood by integrating acoustics and microfluidics* Proc. Natl. Acad. Sci. USA, 114, 10584–10589
Zhang, W., Zhou, X., Zhang, H., Yao, Q., Liu, Y., Dong, Z. (2016) *Extracellular vesicles in diagnosis and therapy of kidney diseases* Am. J. Physiol. Renal Physiol., **311**, F844–F851

Mouse

Phoonsawat, W., Aoki-Yoshida, A., Tsuruta, T. and Sonoyama, K. (2014) *Adiponectin is partially associated with exosomes in mouse serum* Biochem. Biophys. Res. Comm., **448**, 261–266
Shi, M., Liu, C., Cook, T.J., Bullock, K.M. et al (2014) *Plasma exosomal α-synuclein is likely CNS-derived and increased in Parkinson's disease* Acta Neuropathol., **128**, 639–650

Plasma membrane fragments (sheared)

Yoon, J., Jo, W., Jeong, D., Kim, J., Jeong, H. and Park, J. (2015) *Generation of nanovesicles with sliced cellular membrane fragments for exogenous material delivery* Biomaterials, **59**, 12-20

Platelets

Duchez, A-C., Boudreau. L.H., Bollinger, J., Belleannée, C., Cloutier, N., Laffont, B., Mendoza-Villarroel, R.E., Lévesque, T. Rollet-Labelle, E. et al (2015) *Platelet microparticles are internalized in neutrophils via the concerted activity of 12-lipoxygenase and secreted phospholipase A2-IIA* Proc. Natl. Acad. Sci. USA, 112, E3564–E3573
Pienimaeki-Roemer, A., Kuhlmann, K., Böttcher, A., Konovalova, T., Black, A., Orsó, E. Liebisch, G. et al (2015*) Lipidomic and proteomic characterization of platelet extracellular vesicle subfractions from senescent platelets* Transfusion, **55**, 507–521
Sódar, B.W., Kittel, A., Pálóczi, K., Vukman, K.V., Osteikoetxea, X., Szabó-Taylor, K., Németh, A., Sperlágh, B., Baranyai, T. et al (2016) *Low-density lipoprotein mimics blood plasma-derived exosomes and microvesicles during isolation and detection* Sci. Rep., **6**: 24316

Prions/prion diseases

Hartmann, A., Altmeppen, H., Krasemann, S. and Glatzel, M. (2017) *Exosomes in prion diseases* In Prion Diseases, Neuromethods, **129,** (ed. Liberski, P.P.), © Springer Science+Business Media LLC, pp 197-207
Liu, S., Hossinger, A., Göbbels, S. and Vorberg, I.M. (2017) *Prions on the run: How extracellular vesicles serve as delivery vehicles for self-templating protein aggregates* Prion, **11**, 98–112

Prostate carcinoma cells

Hessvik, N.P., Øverbye, A., Brech, A., Torgersen, M.L., Jakobsen, I.S., Sandvig, K. and Llorente, A. (2016) *PIKfyve inhibition increases exosome release and induces secretory autophagy* Cell. Mol. Life Sci. **73**, 4717–4737
Kawakami, K., Fujita, Y., Matsuda, Y., Arai, T., Horie, K., Kameyama, K., Kato, T., Masunaga, K. et al (2017*) Gamma-glutamyltransferase activity in exosomes as a potential marker for prostate cancer* BMC Cancer, **17**: 316
Minciacchi, V.R., You, S., Spinelli, C., Morley, S., Zandian, M., Aspuria, P-J., Cavallini, L., Ciardiello, C., Sobreiro. M.R. et al (2015) *Large oncosomes contain distinct protein cargo and represent a separate functional class of tumor-derived extracellular vesicles* Oncotarget, **6**, 11327-11341
Minciacchi, V., Spinelli, C., Reis-Sobreiro, M., Zandian, M., Adam, R.M., Posadas, E.M., Michael, F.R., Cocucci, E., Bhowmick, N. and Di Vizio, D. (2016) *Large oncosomes reprogram prostate fibroblasts toward a pro-angiogenic phenotype* Cancer Res., **76**, Suppl. 14, Abstr. LB-266
Minciacchi, V.R., Spinelli, C., Reis-Sobreiro, M., Cavallini, L., You, S., Zandian, M., Li, X., Mishra, R., Chiarugi, P., Adam, R.M. et al (2017) *MYC mediates large oncosome-induced fibroblast reprogramming in prostate cancer* Cancer Res., **77**, 2306-2317

Proteomic analysis of exosomes

Hurwitz, S.N. and Meckes, D.G. (2017) *An adaptable polyethylene glycol-based workflow for proteomic analysis of extracellar vesicles* In Extracellular Vesicles: Methods and Protocols, Methods Mol. Biol., **1660**, (ed. Kuo, W.P. and Jia, S.) Springer Science+Business Media, LLC, pp 303-317

Rat-1 cells

Hurwitz, S.N., Nkosi, D., Conlon, M.M., York, S.B., Liu, X., Tremblay, D.C. and Meckes, D.G. (2017) *CD63 regulates Epstein-Barr virus LMP1 exosomal packaging, enhancement of vesicle production, and noncanonical NF-κB signaling* J. Virol., **91**: e02251-16

Renal carcinoma

Horie, K., Kawakami, K., Fujita, Y., Sugaya, M., Kameyama, K., Mizutani, K., Deguchi, T, and Ito, M. (2017) *Exosomes expressing carbonic anhydrase 9 promote angiogenesis* Biochem. Biophys. Res. Comm., **492**, 356-361

Renal tubule cell targeting

Collino, F., Pomatto, M., Bruno, S., Lindoso, R.S., Tapparo, M., Sicheng, W., Quesenberry, P. and Camussi, G. (2017) *Exosome and microvesicle-enriched fractions isolated from mesenchymal stem cells by gradient separation showed different molecular signatures and functions on renal tubular epithelial cells* Stem Cell Rev. Rep., **13**, 226–243

Retinal pigmented epithelial cells

Klingeborn, M., Dismuke, W.M., Skiba, N.P., Kelly, U., Stamer, W.D. and Rickman, C.B. (2017) *Directional exosome proteomes reflect polarity-specific functions in retinal pigmented epithelium monolayers* Sci. Rep., **7**: 4901

Sheep hyatid cysts

Siles-Lucas, M., Sánchez-Ovejero, C., González-Sánchez, M., González, E., Falcón-Pérez J.M., Boufana, B., Fratini, F., Casulli, A. and Manzano-Román, R. (2017) *Isolation and characterization of exosomes derived from fertile sheep hydatid cysts* Vet. Parasitol., **236**, 22–33

siRNA

Lunavat, T.R., Jang, S.C., Nilsson, L., Park, H.T., Repiska, G., Lässer, C., Nilsson, J.A., Gho, Y.S. and Lötvall, J. (2016) *RNAi delivery by exosome-mimetic nanovesicles - Implications for targeting c-Myc in cancer* Biomaterials, **10**, 231-238

Quek, C., Jung, C-h., Bellingham, S.A., Lonie, A. and Hill, A.F. (2015) *ISRAP - a one-touch research tool for rapid profiling of small RNA-seq data* J. Extracell. Vesicles **4**: 29454

Stremersch, S., Vandenbroucke, R.E., VanWonterghemb, E., Hendrix, A., De Smedt, S.C. and Raemdonck, K. (2016) *Comparing exosome-like vesicles with liposomes for the functional cellular delivery of small RNAs* J. Control. Release, **232**, 51–61

Skeletal muscle cells

Le Bihan, M-C., Bigot, A., Jensen, S.S., Dennis, J.L., Rogowska-Wrzesinska, A., Lainé, J., Gache, V., Furling, D., Jensen, O.N., Voita, T., Mouly, V., Coulton, G.R. and Butler-Browne, G. (2012) *In-depth analysis of the secretome identifies three major independent secretory pathways in differentiating human myoblasts* J. Proteom., **77**, 344-356

Stem cells

Collino, F., Pomatto, M., Bruno, S., Lindoso, R.S., Tapparo, M., Sicheng, W., Quesenberry, P. and Camussi, G. (2017) *Exosome and microvesicle-enriched fractions isolated from mesenchymal stem cells by gradient separation showed different molecular signatures and functions on renal tubular epithelial cells* Stem Cell Rev. Rep., **13**, 226–243

Herrera Sanchez, M.B., Previdi, S., Bruno, S., Fonsato, V., Deregibus, M.C., Kholia, S., Petrillo, S., Tolosano, E., Critelli, R. et al (2017) *Extracellular vesicles from human liver stem cells restore argininosuccinate synthase deficiency* Stem Cell Res. Ther., **8**: 176

Kim, H-S., Choi, D-Y., Yun, S.J., Choi, S-M., Kang, J.W., Jung, J.W., Hwang, D., Kim, K.P. and Kim, D-W. (2012) *Proteomic analysis of microvesicles derived from human mesenchymal stem cells* J. Proteome Res., **11**, 839−849

Lai, R.C., Yeo, R.W.Y., Tan, K.H. and Lim, S.K. (2013) *Exosomes for drug delivery — a novel application for the mesenchymal stem cell* Biotechnol. Adv., **31**, 543–551

Lee, J.Y., Kim, E., Choi, S-M., Kim, D-W., Kim, K.P., Lee, I. and Kim, H-S. (2016) *Microvesicles from brain-extract-treated mesenchymal stem cells improve neurological functions in a rat model of ischemic stroke* Sci. Rep., **6**: 33038

Sutaria, D.S., Badawi, M., Phelps, M.A. and Schmittgen, T.D. (2017) *Achieving the promise of therapeutic extracellular vesicles: the devil is in details of therapeutic loading* Pharm. Res., **34**, 1053-1056
Willis, G.R., Kourembanas, S. and Mitsialis, S.A. (2017) *Therapeutic applications of extracellular vesicles: perspectives from newborn medicine in extracellular vesicles* In Methods and Protocols, Methods Mol. Biol., **1660**, (ed. Kuo, W.P. and Jia, S.) Springer Science+Business Media, LLC, pp 409-432

Suprachiasmatic nuclei cells

Soetedjo, L. and Jin, H. (2014) *Agonist-induced GPCR shedding from the ciliary surface is dependent on ESCRT-III and VPS4* Curr., Biol., **24**, 509-518

Synovial fluid and synoviocytes (fibroblast-like)

Boere, J., van de Lest, C.H.A., Libregts, S.F.W.M., Arkesteijn, G.J.A., Geerts, W.J.C., Nolte-'t Hoen, E.N.M., Malda, J., van Weeren, P.R. and Wauben, M.H.M. (2016) *Synovial fluid pretreatment with hyaluronidase facilitates isolation of CD44+ extracellular vesicles* J. Extracell. Vesicles, **5**: 31751
Edhayan, G., Ohara, R.A., Stinson, W.A., Amin, M.A., Isozaki, T., Ha, C.M., Haines III, K., Morgan, R. et al (2016) *Inflammatory properties of inhibitor of DNA binding 1 secreted by synovial fibroblasts in rheumatoid arthritis* Arthritis Res. Ther., **18**: 87
Morgan, R.L., Behbahani-Nejad, N., Endres, J., Amin, M.A., Lepore, N.J., Du, Y., Urquhart, A., Chung, K.C. and Fox, D.A. (2016) *Localization, shedding, regulation and function of aminopeptidase N/CD13 on fibroblast like synoviocytes* PloS One, **11**: e0162008

Trabecular meshwork cells

Dismuke, W.M., Klingeborn, M. and Stamer, W.D. (2016) *Mechanism of fibronectin binding to human trabecular meshwork exosomes and its modulation by dexamethasone* PLoS One, **11**: e0165326

Trophoblasts

Ouyang, Y., Bayer, A., Chu, T., Tyurin, V., Kagan, V., Morelli, A.E., Coyne, C.B. and Sadovsky, Y. (2016) *Isolation of human trophoblastic extracellular vesicles and characterization of their cargo and antiviral activity* Placenta, **47**, 86-95
Truong, G., Guanzon, D., Kinhal, V., Elfeky, O., Lai, A., Longo, S., Nuzhat, Z., Palma, C. et al (2017) *Oxygen tension regulates the miRNA profile and bioactivity of exosomes released from extravillous trophoblast cells - liquid biopsies for monitoring complications of pregnancy* PLoS One, **12**: e0174514

Tumour epithelial cells

Sanchez, V.C., Craig-Lucas, A., Wei, B-R., Shukla, A., Read, A., Lou, J., Simpson, M., Hunter, K. and Yuspa, S. (2017) *Chloride intracellular channel protein 4 (CLIC4) is a serological cancer biomarker released from tumour epithelial cells via extracellular vesicles* J. Extracell. Vesicles Abstr. PF01.12

Urinary

Fraser, K.B., Moehle, M.S., Daher, J.P.L., Webber, P.J., Williams, J.Y., Stewart, C.A., Yacoubian, T.A., Cowell, R.M., Dokland, T., Ye, T. et al (2013) *LRRK2 secretion in exosomes is regulated by 14-3-3* Hum. Mol. Genet., **22**, 4988–5000
Dhondt, B., Vergauwen, G., Van Deun, J., Geeurickx, E., Claeys, T., Poelaert, F., Buelens, S., Hendrix, A., De Wever, O. and Lumen, N. (2017) *Purification of urinary extracellular vesicles for uro-oncological biomarker studies using an iodixanol (Optiprep™) density gradient* Eur. Urol. Suppl., **16**, e1078

Urogenital cancers

Nawaz, M., Camussi, G., Valadi, H., Nazarenko, I., Ekström, K., Wang, X. et al (2014) *The emerging role of extracellular vesicles as biomarkers for urogenital cancers* Nat. Rev. Urol., **11**, 688–701

4 Electroporation of exosomes

The use of exosomes to introduce into target cells, molecules that have been artificially inserted by electroporation, is being investigated as potential treatment for a number of diseases. The methodology involves the selection of cells, such as dendritic cells, that are engineered to express an exosomal protein linked an organ-specific peptide (e.g. the neuron-specific RVG peptide). siRNA drugs are then introduced into the exosomes by electroporation, which can thus be targeted to a specific organ (the brain in the case of the RVG peptide). This technology may be viewed as a potential means of controlling, for example in the case of the RVG peptide, Alzheimer's disease. Refs 1-7 presented the methods that might be used for introducing useful molecules.

References to Section 4

1. **Alvarez-Erviti, L.**, Seow, Y., Yin, H-F., Betts, C., Lakhal, S. and Wood, M.J.A. (2011) *Delivery of siRNA to the mouse brain by systemic injection of targeted exosomes* Nat. Biotech., **4**, 341-345
2. **El-Andaloussi, S.**, Lee, Y., Lakhal-Littleton, S., Li, J., Seow, Y., Gardiner, C., Alvarez-Erviti, L., Sargent, I.L. and Wood, M.J.A. (2012) *Exosome-mediated delivery of siRNA in vitro and in vivo* Nat. Protocols, **7**, 2112-2126
3. **Kamerkar, S.**, LeBleu, V.S., Sugimoto, H., Yang, S., Ruivo, C.F., Melo, S.A., Lee, J.J. and Kalluri, R. (2017) *Exosomes facilitate therapeutic targeting of oncogenic KRAS in pancreatic cancer* Nature 546, 498-503
4. **Kooijmans, S.A.A.**, Stremersch, S., Braeckmans, K., de Smedt, S.C., Hendrix, A., Wood, M.J.A., Schiffelers, R.M., Raemdonck, K. and Vader, P. (2013) *Electroporation-induced siRNA precipitation obscures the efficiency of siRNA loading into extracellular vesicles* J. Control. Release, **172**, 229-238
5. **Lamichhane, T.N.**, Raiker, R.S. and Jay, S.M. (2015) *Exogenous DNA loading into extracellular vesicles via electroporation is size-dependent and enables limited gene delivery Mol. Pharmaceut.* ***12**, 3650-3657*
6. **Lunavat, T.R.**, Jang, S.C., Nilsson, L., Park, H.T., Repiska, G., Lässer, C., Nilsson, J.A., Gho, Y.S. and Lötvall, J. (2016) *RNAi delivery by exosome-mimetic nanovesicles - Implications for targeting c-Myc in cancer* Biomaterials, **10**, 231-238
7. **Yang, Z.**, Xie, J., Zhu, J., Kang, C., Chiang, C., Wang, X., Wang, X., Kuang, T., Chen, F. et al (2016) *Functional exosome-mimic for delivery of siRNA to cancer: in vitro and in vivo evaluation* J. Control. Release, **243**, 160-171

5. Reviews of methodology, exosome function and clinical applications

Abramowicz, A., Widlak, P. and Pietrowska, M. (2016) *Proteomic analysis of exosomal cargo: the challenge of high purity vesicle isolation* Mol. BioSyst., **12**, 1407-1419

Bellingham, S.A., Guo, B. and Hill, A.F. (2015) *The secret life of extracellular vesicles in metal homeostasis and neurodegeneration* Biol. Cell, **107,** 389–418

Chaput, N. and Théry, C. (2011) *Exosomes: immune properties and potential clinical implementations* Semin. Immunopathol., **33**, 419–440

Choi, D-S., Kim, D-K., Kim, Y-K. and Gho, Y.S. (2013) *Proteomics, transcriptomics and lipidomics of exosomes and ectosomes* Proteomics, **13**, 1554–1571

Costa, J. (2017) *Glycoconjugates from extracellular vesicles: structures, functions and emerging potential as cancer biomarkers* Rev. Cancer, **1868**, 157–166

Fleming, A., Sampey, G., Chung, M-C., Bailey, C., van Hoek, M.L., Kashanchi, F. and Hakami, R.M. (2014) *The carrying pigeons of the cell: exosomes and their role in infectious diseases caused by human pathogens* Pathog. Dis., **71**, 107–118

Fontana, S., Giallombardo, M. and Alessandro, R. (2017) *Technical aspects for the evaluation of exosomes and their content* In Liquid Biopsy in Cancer Patients, Current Clinical Pathology (ed. Giordano et al) © Springer International Publishing AG, pp 61-70

Gholizadeha, S., Draz, M.S., Zarghoonic, M., Sanati-Nezhadd, A., Ghavamie, S., Shafiee, H. and Akbarih, M. (2017) *Microfluidic approaches for isolation, detection, and characterization of extracellular vesicles: Current status and future directions* Biosens. Bioelectron., **91**, 588–605

Gopal, S.K., Greening, D.W., Rai, A., Chen, M., Xu, R., Shafiq, A., Mathias, R.A., Zhu, H-J. and Simpson, R.J. (2017) *Extracellular vesicles: their role in cancer biology and epithelial–mesenchymal transition* Biochem. J., **474**, 21–45

György, B., Szabó, T.G., Pászto´I, M., Pál, Z., Misják, P., Aradi, B., László, V., Pállinger, E., Pap, E., Kittel, A., Nagy, G., Falus, A. and Buzás, E.I. (2011) *Membrane vesicles, current state-of-the-art: emerging role of extracellular vesicles* Cell. Mol. Life Sci., **68**, 2667–2688

Ha, D., Yang, N. and Nadithen, V. (2016) *Exosomes as therapeutic drug carriers and delivery vehicles across biological membranes: current perspectives and future challenges* Acta Pharmaceutica Sinica B, **6**, 287-296

Hagiwara, K., Ochiya, T. and Kosaka, N. (2014) *A paradigm shift for extracellular vesicles as small RNA carriers: from cellular waste elimination to therapeutic applications* Drug Deliv. Transl. Res., **4**,:31−37

Han, L., Xu, J., Xu, Q., Zhang, B., Lam, E.W-F. and Sun, Y. (2017) *Extracellular vesicles in the tumor microenvironment: Therapeutic resistance, clinical biomarkers, and targeting strategies* Med. Res. Rev., **37**, 1318–1349

Kang, H., Kim, J. and Park, J. (2017) *Methods to isolate extracellular vesicles for diagnosis* Micro Nano Syst. Lett., **5**: 15

Kanninen, K.M., Bister, N., Koistinaho, J. and Malm, T. (2016) *Exosomes as new diagnostic tools in CNS diseases* Biochim. Biophys. Acta, **1862**, 403–410
Kim, O.Y., Lee, J. and Gho, Y.S. (2017) *Extracellular vesicle mimetics: Novel alternatives to extracellular vesicle-based theranostics, drug delivery, and vaccines* Semin.Cell Dev. Biol., **67**, 74–82
Kinoshita, T., Yip, K.W., Spence, T. and Liu, F-F. (2017) *MicroRNAs in extracellular vesicles: potential cancer biomarkers* J. Hum. Genet., **62**, 67–74
Kreimer, S., Belov, A.M., Ghiran, I., Murthy, S.K., Frank, D.A. and Ivanov, A.R. (2015) *Mass-spectrometry-based molecular characterization of extracellular vesicles: lipidomics and proteomics* J. Proteome Res., **14**, 2367−2384
Lane, R.E., Korbie, D., Anderson, W., Vaidyanathan, R. and Trau, M. (2015) *Analysis of exosome purification methods using a model liposome system and tunable-resistive pulse sensing* Scientific Rep., **5**: 7639
Lane, R.E., Korbie, D., Trau, M. and Hill, M.M. (2017) *Purification protocols for extracellular* vesicles In Extracellular Vesicles: Methods and Protocols, Methods Mol. Biol., **1660**, (ed. Kuo, W.P. and Jia, S.) Springer Science+Business Media, LLC, pp 111-130
Lindner, K., Haier, J., Wang, Z., Watson, D.I., Hussey, D.J. and Hummel, R. (2015) *Circulating microRNAs: emerging biomarkers for diagnosis and prognosis in patients with gastrointestinal cancers* Clin. Sci., **128**, 1–15
Lu, M., Xing, H., Yang, Z., Sun, Y., Yang, T., Zhao, X., Cai, C., Wang, D. and Ding, P. (2017) *Recent advances on extracellular vesicles in therapeutic delivery: Challenges, solutions, and opportunities* Eur. J. Pharmaceut. Biopharmaceut., **119**, 381–395
Mathivanan, S., Ji, H. and Simpson, R.J. (2010) *Exosomes: extracellular organelles important in intercellular communication* J. Proteomics, **73**, 1907-1920
Miller, I.V. and Grunewald, T.G.P. (2015) *Tumour-derived exosomes: Tiny envelopes for big stories* Biol. Cell, **107**, 287–305
Momen-Heravi, F. (2017) *Isolation of extracellular vesicles by ultracentrifugation* In Extracellular Vesicles: Methods and Protocols, Methods Mol. Biol., **1660**, (ed. Kuo, W.P. and Jia, S.) Springer Science+Business Media, LLC, pp 25-32
Moore, C., Kosgodage, U., Lange, S. and Inal, J.M. (2017) *The emerging role of exosome and microvesicle-(EMV-) based cancer therapeutics and immunotherapy* Int. J. Cancer, **141**, 428–436
Osteikoetxea, X., Németh, A., Sódar, B.W., Vukman, K.V. and Buzás, E.I. (2016) *Extracellular vesicles in cardiovascular disease: are they Jedi or Sith?* J. Physiol., **594**, 2881–2894
Raimondo, F., Morosi, L., Chinello, C., Magni, F. and Pitto, M. (2011) *Advances in membranous vesicle and exosome proteomics improving biological understanding and biomarker discovery* Proteomics, **11**, 709–720
Reiner, A.T., Witwer, K.W., Van Balkom, B.W.M., De Beer, J., Brodie C., Corteling, R.L., Gabrielsson, S., Gimona, M., Ibrahim, A.G. et al (2017) *Concise Review: Developing best-practice models for the therapeutic use of extracellular vesicles* Stem Cells Translat. Med., **6**, 1730–1739
Revenfeld, A.L.S., Bæk, R., Nielsen, M.H., Stensballe, A., Varming, K. and Jørgensen, M. (2014) *Diagnostic and prognostic potential of extracellular vesicles in peripheral blood* Clin. Ther., **36**, 830-84
Rupert, D.L.M., Claudio, V., Lässer, C. and Bally, M. (2017) *Methods for the physical characterization and quantification of extracellular vesicles in biological samples* Biochim. Biophys. Acta, **1861**, 3164–3179
Safdar, A., Saleem, A. and Tarnopolsky, M.A. (2016) *The potential of endurance exercise-derived exosomes to treat metabolic diseases* Nature Rev., **12**, 505-517
Simpson, R.J. (2017) *Extracellular vesicles* Semin. Cell Devel. Biol., **67**, 1–2
Skalnikova, H.K. (2013) *Proteomic techniques for characterisation of mesenchymal stem cell secretome* Biochimie, **95**, 2196-2211
Steinbichler, T.B., Dudás, J., Riechelmann, H. and Skvortsova, I-I., (2017) *The role of exosomes in cancer metastasis* Semin. Cancer Biol., **44**, 170–181
Stremersch, S., De Smedt, S.C. and Raemdonck, K. (2016) *Therapeutic and diagnostic applications of extracellular vesicles* J.ournal of Control. Release, **244**, 167–183
Strotman, L.N. and Linder, M.W. (2016) *Extracellular vesicles move toward use in clinical laboratories* Clin. Lab. Med., **36**, 587–602
Sutaria, D.S., Badawi, M., Phelps, M.A. and Schmittgen, T.D. (2017) *Achieving the promise of therapeutic extracellular vesicles: the devil is in details of therapeutic loading* Pharm. Res., **34**, 1053-1056
Syn, N.L., Wang, L., Kai-Hua Chow, E., Teck Lim, C. and Goh, B-C., (2017) *Exosomes in cancer nanomedicine and immunotherapy: prospects and challenges* Trends Biotech., 35, 665-676
Tamura, R. and Yin, H. (2017) *Rationally-designed peptide probes for extracellular vesicles* Adv. Clin, Chem., **79**, 25-41
Taylor, D.D. and Shah, S. (2015) *Methods of isolating extracellular vesicles impact down-stream analyses of their cargoes* Methods, **87,** 3–10
Urabe, F., Kosaka, N., Yoshioka, Y., Egawa, S. and Ochiya, T. (2017) *The small vesicular culprits: the investigation of extracellular vesicles as new targets for cancer treatment* Clin. Trans. Med., **6**: 45

Wu, K., Xing, F., Wu, S-Y. and Watabe, K. (2017) *Extracellular vesicles as emerging targets in cancer: Recent development from bench to bedside* BBA – Rev. Cancer, **1868**, 538–563
Zaborowski, M.J.P., Balaj, L., Breakefield, X.O. and Lai, C.P. (2015) *Extracellular vesicles: composition, biological relevance, and methods of study* BioScience **65**, 783–797
Zhang, W., Zhou, X., Zhang, H., Yao, Q., Liu, Y., Dong, Z. (2016) *Extracellular vesicles in diagnosis and therapy of kidney diseases* Am. J. Physiol. Renal Physiol., **311**, F844–F851
Zheng, X., Chen, F., Zhang, J., Zhang, Q. and Lin, J. (2014) *Exosome analysis: a promising biomarker system with special attention to saliva* J. Membrane Biol., **247,** 1129–1136
Zocco, D. and Zarovni, N. (2017) *Extraction and analysis of extracellular vesicle-associated miRNAs following antibody-based extracellular vesicle capture from plasma samples* In Extracellular Vesicles: Methods and Protocols, Methods Mol. Biol., **1660**, (ed. Kuo, W.P. and Jia, S.) Springer Science+Business Media, LLC, pp 269-285

Mini-Review MS13; 3rd edition, February 2018

Alere Technologies AS

Axis-Shield Density Gradient Media
is a brand of Alere Technologies AS