OptiPrep™ Mini-Review MS13

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

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BHK cells

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Blood – see Plasma

Bone marrow mast cells

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Bone marrow mesenchymal stromal cells

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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

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Cerebro-spinal fluid

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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

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

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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

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Embryonic stem cells – see also "Stem cells"

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Epididymosomes

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Epstein Barr virus LMP1

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Erythrocytes

Equine anaemia

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Plasmodium-infected

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Exosome profiling

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Exosome size

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Exosomes *versus* microvesicles

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Head and neck cancers

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Hepatocytes

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Herpes simplex virus infection

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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

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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.

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