OptiPrep™ Mini-Review MS17

Eukaryotic cell exosomes and other microvesicles from conditioned medium, an analytical review

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 MS10 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 MS16 and Application Sheet S60

♦ Other Mini-Reviews or OptiPrep™ Application Sheets can be accessed on the 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 Sheets 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.
3. 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.
4. 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. A short addendum, Section 3a is devoted to electroporation of exosomes.**

- ♦ **Reviews of the methodology and exosome function are listed in Section 4 of this Mini-Review**

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

References are listed according to tissue/cell type and **first author**). Important research topics are highlighted in blue in the titles

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

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

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 carcinoma

Baietti, M.F., Zhang, Z., Mortier, E., Melchior, A., Degeest, G., Geeraerts, A., Ivarsson, Y., Depoortere, F., Coomans, C., Vermeiren, E., Zimmermann, P. and David, G. (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

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Cell, plasma membrane fragments

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

Colon carcinoma (see also “Gastrointestinal cancers”)

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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

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

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Drug delivery

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

Endometrial stromal cells

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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

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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

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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

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Exosome function in tumours

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Fibrosarcoma cells

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Gene delivery

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HEK cells

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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

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Hepatitis C virus particles

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Herpes simplex virus infection

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HIV-RNA

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Human hepatic cells

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Human plasma

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Human trophoblast

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Hypothalamus

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Insects

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Lung cancer cells

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Macrophages (see "Monocytes")

Mast cells

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3a 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 1-6 presented the methods that might be used for introducing useful molecules.

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