

OptiPrep™ Mini-Review MS14

Extracellular vesicles from non-mammalian sources

1. Introduction

It is widely recognized that mammalian cells, bacteria, algae and fungi release extracellular vesicles into the surrounding medium; these vesicles are involved in communication between cells and the delivery of biologically and clinically important molecules to other cells. With regard to bacteria the term “extracellular vesicles” (EVs) covers the outer membrane vesicles (OMVs) produced by Gram-negative bacteria and the membrane vesicles (MVs) produced by Gram-positive bacteria and other organisms such as algae and fungi. In all cases; EVs are distinct from the intracellular vesicles present in the cytoplasm. The OMVs from Gram-negative bacteria 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.

- ◆ **Section 2** briefly reviews the current OptiPrep™-based methodology; this is described in detail in **Application Sheet S60**. It has its own short reference list.
- ◆ The principal aim of this Mini-Review however is to provide a bibliography of all the published papers that have reported the use of an iodixanol gradient.
- ◆ **Section 3a is devoted to Gram –ve bacteria**
- ◆ **Section 3b to Gram +ve bacteria and mycobacteria**
- ◆ **Section 3c to Biofilm structures**
- ◆ **Section 3d to mycoplasmas**
- ◆ **Section 3e to protozoa, algae and fungi**
- ◆ **Section 3f to insects**
- ◆ **Section 3g to trematodes**
- ◆ **Section 3h to plants**
- ◆ **Section 3i contains references to methodological reviews**
- ◆ **All references are listed alphabetically according to first author**

Related research areas that have reported the use of gradients prepared from OptiPrep™ are:

- ◆ The analysis of the microvesicles that are expressed from the surface of mammalian cells is covered in **OptiPrep™ Mini-Review MS13 and OptiPrep™ Application Sheet S61**
- ◆ The control and organization of membrane trafficking within mammalian cells that permits the movement of vesicles to, and ultimately their fusion with, the plasma membrane or a specific plasma membrane domain is covered in **Optiprep™ Mini-Review MS12 and OptiPrep™ Application Sheet S45**
- ◆ These other Mini-Reviews or OptiPrep™ Application Sheets can be accessed from the OptiPrep™ flash drive or from the following website: www.axis-shield-density-gradient-media.com, click on “Mini-Reviews” or “Methodology”. On the flash drive return to “Mini-Reviews” or “Application Sheets”.

2. Methodological summary

Various forms of pre-gradient processing are employed, during which intact bacteria and aggregated material in the culture medium are mostly removed and the EVs concentrated. This is covered in much greater detail in **Application Sheet S60**

The first step is clarification of the bacterial broth to remove intact cells by centrifugation; the time and *g*-force used varies widely and reflects the size of the organism. Generally *rcfs* of approx. 10,000 *g* are used for 10-20 min, but there are examples both of lower and higher *g*-forces so that the range spans 4,000-12,000 *g*. Occasionally this first step is carried out in two stages in which the broth is centrifuged for 30 min at 5,000 *g* and then the supernatant at 7,500 *g*. This strategy may minimize the

entrapment (and consequent loss) of vesicles into the pellet by the rapidly sedimenting much larger bacteria. Fungal cells appear to sediment satisfactorily at lower speeds such as 2,500 g for 10 min. **Commonly the second step is volume reduction** since ultimately the vesicles will be sedimented in a fixed-angle ultracentrifuge rotor, prior to gradient purification. Large volumes of clarified medium can pose a problem for this final pre-gradient step. The Beckman 45Ti for example which accommodates 6x94 ml tubes has a maximum *g*-force of 235,000 g, but the vesicles at the top of the sample experience initially only 80,000 g. Tangential filtration devices (e.g. 70-100 kDa cut-off) are popular or centrifugal filters are popular for this volume reduction step.

Residual bacteria in the broth may then be removed by vacuum filtration through either a 0.45 or 0.22 µm filter or both pore size filters sequentially, before the EVs are sedimented in a fixed-angle rotor; the conditions vary quite widely, from approx. 40,000 g for 1 h to 140-150,000 g for 2-3 h..

Ammonium sulphate precipitation of OMVs from the clarified broth of Gram-ve bacteria has been used a few cases. The precipitation process may only require about half an hour at 4°C but can be as long as overnight. Moreover the resuspended pellet requires dialysis overnight prior to further processing.

Iodixanol gradient purification of the EVs, regardless of the pre-gradient technology, involves adjustment of the suspension to a density of approx. 1.241 g/ml (sometimes higher 1.267 g/ml or lower 1.215 g/ml).for subsequent flotation through a discontinuous iodixanol gradient; During centrifugation at 100-200,000 g for 12-16 h the gradient will become more or less continuous; gradients run for shorter times (e.g. 2 h) will retain some of their discontinuous nature.

The density of MVs in iodixanol gradients is generally >1.11 g/ml, many banding between 1.13 and 1.15 g/ml; although occasionally densities of as high as 1.20 g/ml have been observed. There is also evidence of heterogeneity amongst EV populations from a single bacterial type.

The first published paper to describe the use of OptiPrep™, of which we have a record, was by Horstman and Kuehn, published in 2000. It documented the purification of OMVs from enterotoxin-containing *Escherichia coli*; the OMVs were shown to contain the pathogenic enterotoxin, lipids and specific proteins characteristic of the outer membrane and proteins from the periplasmic space, but no markers of the cytosol or inner membrane ([see Section 3a - Escherichia coli](#)).

3. Bibliography of publications reporting analytical studies on iodixanol-purified OMVs and MVs

References are sorted alphabetically according to first author. [Research topics are highlighted in blue in the titles](#)

3a. Gram-negative bacteria

Acinetobacter baumannii

[Chatterjee, S., Mondal, A., Mitra, S. and Basu, S. \(2017\) *Acinetobacter baumannii* transfers the *bla*_{NDM-1} gene via outer membrane vesicles](#) J. Antimicrob. Chemother., **72**, 2201–2207

Aggregatibacter actinomycetemcomitans

[Kieselbach, T. and Oscarsson, J. \(2017\) Dataset of the proteome of purified outer membrane vesicles from the human pathogen *Aggregatibacter actinomycetemcomitans*](#) Data in Brief, **10**, 426-431

[Rompikuntal, P.K., Thay, B., Khan, M.K., Alanko, J., Penttinen, A-M., Asikainen, S., Wai, S.N. and Oscarsson, J. \(2012\) *Perinuclear localization of internalized outer membrane vesicles carrying active cytolethal distending toxin from *Aggregatibacter actinomycetemcomitans**](#) Infect. Immun., **80**, 31-42

Alteromonas

[Billler, S.J., McDaniel, L.D., Breitbart, M., Rogers, E., Paul, J.H. and Chisholm, S.W. \(2017\) Membrane vesicles in sea water: *heterogeneous DNA content and implications for viral abundance estimates*](#) ISME J., **11**, 394–404

Borrelia burgdorferi

- Coleman, J.L.**, Crowley, J.T., Toledo, A.M. and Benach, G.L. (2013) *The HtrA protease of Borrelia burgdorferi degrades outer membrane protein BmpD and chemotaxis phosphatase CheX* Mol. Microbiol., **88**, 619–633
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- Toledo, A.**, Coleman, J.L., Kuhlow, C.J., Crowley, J.T. and Benach, J.L. (2012) *The enolase of Borrelia burgdorferi is a plasminogen receptor released in outer membrane vesicles* Infect. Immun., **80**, 359–368

Burkholderia glumae

- Kang, Y.**, Goo, E., Kim, J. and Hwang, I. (2017) *Critical role of quorum sensing-dependent glutamate metabolism in homeostatic osmolality and outer membrane vesiculation in Burkholderia glumae* Sci. Rep., **7**: 44195

Burkholderia pseudomallei

- Nieves, W.**, Heang, J., Asakrah, S., Höner zu Bentrup, K., Roy, C.J. and Morici, L.A. (2010) *Immuno-specific responses to bacterial elongation factor Tu during Burkholderia infection and immunization* PloS One **5**: e14361

Campylobacter jejuni

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Escherichia coli

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Francisella novicida

McCaig, W.D., Koller, A. and Thanassi, D.G. (2013) *Production of outer membrane vesicles and outer membrane tubes by Francisella novicida* J. Bacteriol., **195**, 1120-1132

Francisella tularensis

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Haemophilus influenzae

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Haemophilus parasuis

McCaig, W.D., Loving, C.L., Hughes, H.R. and Brockmeier, S.L. (2016) *Characterization and vaccine potential of outer membrane vesicles produced by Haemophilus parasuis* PLoS One **11**: e0149132

Helicobacter pylori

Choi, H-I., Choi, J-P., Seo, J., Kim, B.J., Rho, M., Han, J.K. and Kim, J.G. (2017) *Helicobacter pylori*-derived extracellular vesicles increased in the gastric juices of gastric adenocarcinoma patients and induced inflammation mainly via specific targeting of gastric epithelial cells Exp. Mol. Med., **49**, e330

Klebsiella pneumoniae

Cahill, B.K., Seeley, K.W., Gutel, D. and Ellis, T.N. (2015) *Klebsiella pneumoniae O antigen loss alters the outer membraneprotein composition and the selective packaging of proteins into secreted outer membrane vesicles* Microbiol. Res., **180**, 1–10

Legionella pneumophila

Fernandez-Moreira, E., Helbig, J.H. and Swanson, M.S. (2006) *Membrane vesicles shed by Legionella pneumophila inhibit fusion of phagosomes with lysosomes* Infect. Immun., **74**, 3285-3295

Marinobacter guineae

See “*Shewanella livingstonensis*”

Myobacteria

See Section 3b

Neisseria gonorrhoeae

Pérez-Cruz, C., Delgado, L., López-Iglesias, C. and Mercade, E. (2015) *Outer-inner membrane vesicles naturally secreted by Gram-negative pathogenic bacteria* PLoS One, **10**: e0116896

Neisseria meningitidis

Matthias, K.A., Strader, M.B., Nawar, H.F., Gao, Y.S., Lee, J., Patel, D.S., Im, W. and Bash, M.C. (2017) *Heterogeneity in non-epitope loop sequence and outer membrane protein complexes alters antibody binding to the major porin protein PorB in serogroup B Neisseria meningitidis* Mol. Microbiol., **105**, 934–953

Porphyromonas gingivalis

Cecil, J.D., O’Brien-Simpson, N.M., Lenzo, J.C., Holden, J.A., Chen, Y-Y., Singleton, W., Gause, K.T., Yan, Y., Caruso, F. and Reynolds, E.C. (2016) *Differential responses of pattern recognition receptors to outer membrane vesicles of three periodontal pathogens* PLoS One **11**: e0151967

Prochlorococcus

Billar, S.J., McDaniel, L.D., Breitbart, M., Rogers, E., Paul, J.H. and Chisholm, S.W. (2017) *Membrane vesicles in sea water: heterogeneous DNA content and implications for viral abundance estimates* ISME J., **11**, 394–404

Pseudoalteromona

See “*Shewanella livingstonensis*”

Pseudomonas aeruginosa

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***Pseudomonas panacis* (from faeces)**

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Psychrobacter fozii

See “*Shewanella livingstonensis*”

Salinicola

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Tannerella forsythia

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Thalassospria

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Treponema denticola

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Vibrio cholerae

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