

The impact of *Fusobacterium nucleatum*-derived outer membrane vesicles at the intestinal mucosa

Aizhan Uteubayeva (ID 100340929)

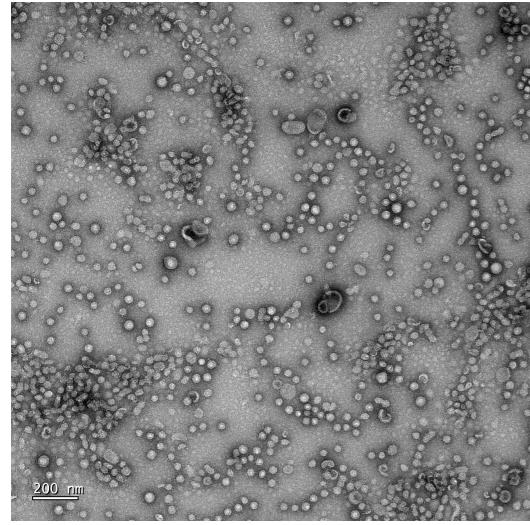


Figure 1. Electron microscopy image of OMVs from *F.nucleatum* ATCC 25586 strain (1).

Abstract

F.nucleatum, a gram-negative bacteria producing outer membrane vesicles is known to impact immune and intestinal function. Here, the OMVs average size of 90-110 nm is shown amongst strains (ATCC 10953, 25586, 51191) and internalised by intestinal organoids. A future comparative genomic analysis with other gram-negative bacteria shown to have OMVs being uptake will elucidate potential mechanisms.

Introduction

Fusobacterium nucleatum (*F.nucleatum*) is a gram-negative bacteria which strains produce outer membrane vesicles (OMVs) (Figure 1). The outer membrane vesicles modulate the host immune response and gut barrier function (2). The impact and mechanism of *F.nucleatum* OMVs internalization is yet to be discovered.

Hypothesis: *F.nucleatum*-derived OMVs are internalised by colonic epithelial cells, the mechanism of which is similar to other gram-negative bacteria the outer membrane vesicles of which are known to be internalised by host epithelial cells.

Aim: to identify *F.nucleatum* OMVs internalization following by the comparative genomic data-analysis with other gram-negative bacteria OMVs.

Method

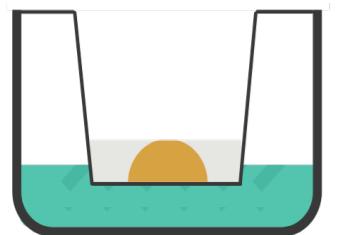
Part 1: OMV Purification

- Bacterial culture
- Filtration of pellet
- Ultra-centrifuging
- Separation by gradient
- Nanosize machine



Part 2: Intestinal Organoids

- Organoids derived from human biopsies
- Cultured on trans-wells
- Incubate with stained OMVs for 24 hours

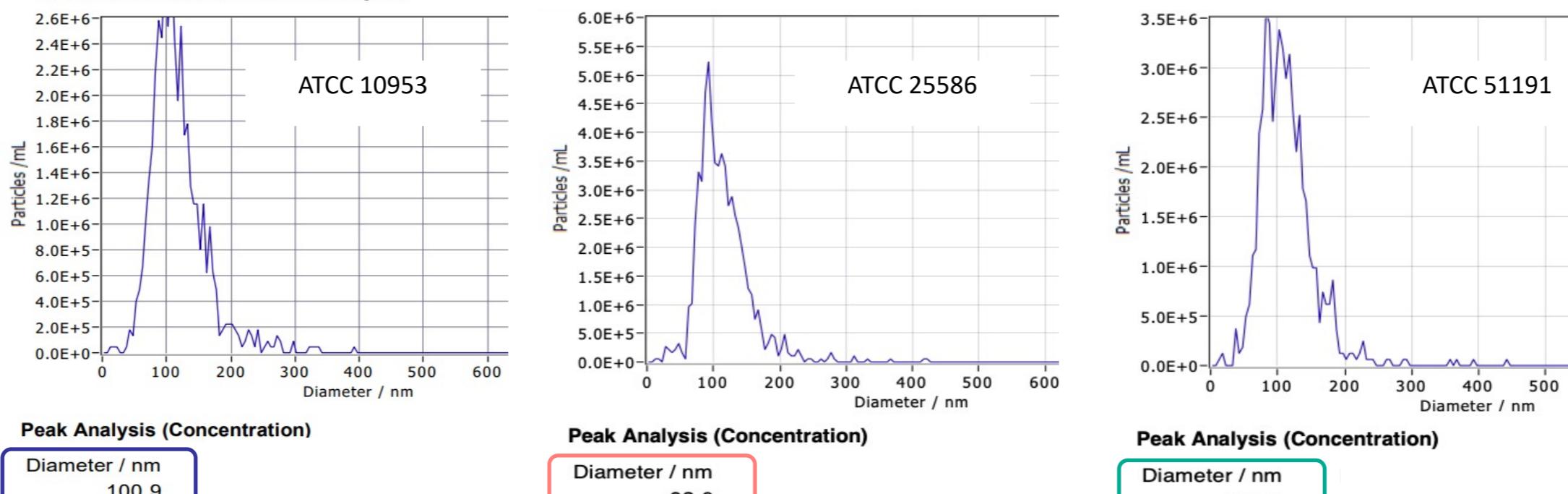


Part 3: Data-analysis

The genomic data from other gram-negative bacteria which OMVs are known to be internalised is compared with *F.nucleatum* and visualised.

Results I

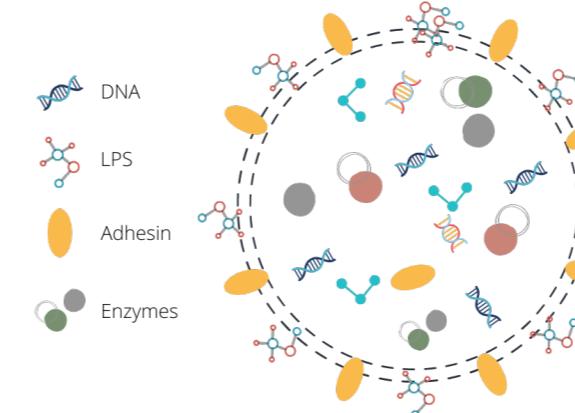
The average size of *F.nucleatum* OMVs is 90-110 nm



Results III

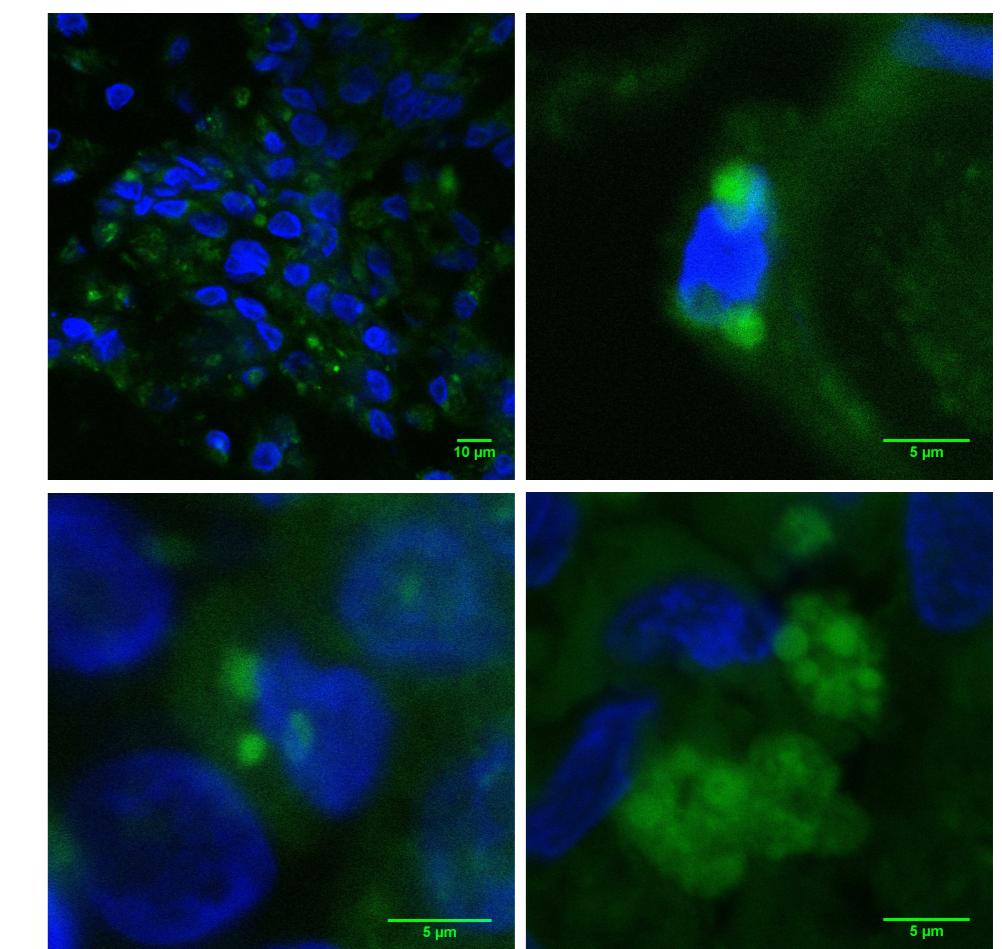
Genomic data-analysis with gram- OMVs

Vesicles are known to comprise of adhesin molecules, lipopolysaccharides (LPS) on the outside; DNA and enzymes on the inside (2). The composition can be similar to other gram-negative bacteria OMVs the mechanism of internalisation of which is elucidated may suggest overlapping mechanisms with *F.nucleatum*.



Results II

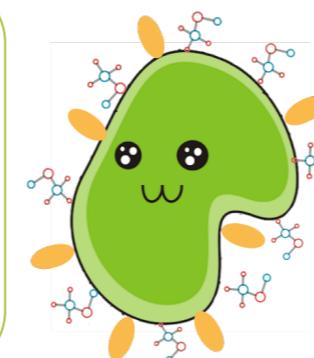
OMVs are internalised by organoids



Confocal microscopy images of intestinal organoids (nucleus labeled in blue) on trans-well incubated for 24 hours with *F.nucleatum* ATCC 25586 OMVs (labeled in green).

Conclusion

Fusobacterium nucleatum outer membrane vesicles are internalized by colonic epithelial cells. Further genomic analysis of *F. nucleatum* and comparison with other gram-negative bacterial OMVs may suggest overlapping mechanisms in interference with intestinal mucosa.



References:

- Unpublished
- Wu, Y., et al., 2018. *Fusobacterium nucleatum* Potentiates Intestinal Tumorigenesis in Mice via a Toll-Like Receptor 4/p21-Activated Kinase 1 Cascade. *Dig Dis Sci* 63, 1210–1218.
- Ellis, T.N., Kuehn, M.J., 2010. Virulence and Immunomodulatory Roles of Bacterial Outer Membrane Vesicles. *Microbiol. Mol. Biol. Rev.* 74, 81–94.

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