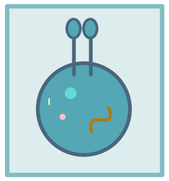
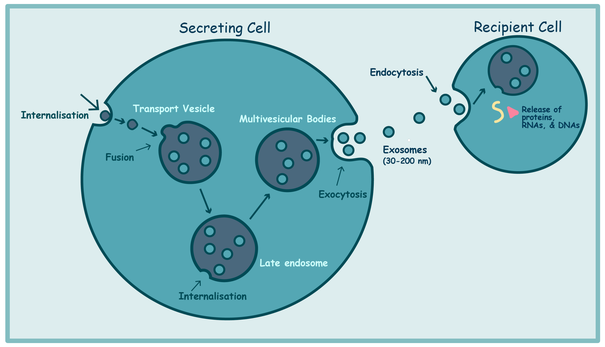
The Role of Extracellular Vesicles in the Pathogenesis of Diabetic Retinopathy

Valeria M. Diaz and Hu Huang
Division of Biological Sciences, University of Missouri School of Medicine, Columbia MO 65201

Exosomes and Biogenesis Pathway

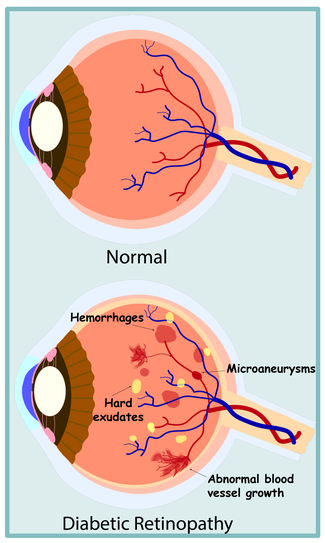


Exosomes, are double-membrane extracellular vesicles (EV) produced by almost every cell type, mainly from the reverse budding of multivesicular bodies. They range from 30-150 nm in size and carry lipids, proteins, DNAs. and RNAs.

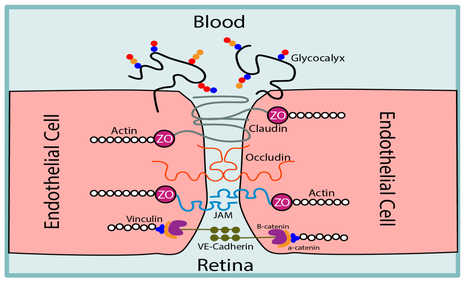


Exosomes are produced through the endocytic pathway. Membrane proteins and lipid complexes are internalized into vesicles and fused, forming late endosomes and MVBs through exocytosis.

Diabetic Retinopathy and Blood-Retinal Barrier



Diabetic retinopathy (DR) is a major complication of diabetes mellitus (DM) and remains a leading cause of visual loss in working-age populations. Clinically, DR is divided into two stages: *non-proliferative* diabetic retinopathy (NPDR) and *proliferative* diabetic retinopathy (PDR).



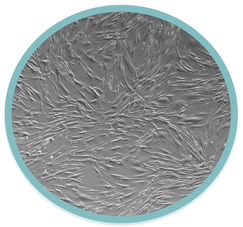
The *blood retinal barrier* is made up of tight junctions (Claudin, Occludin, and JAM linked to ZO-1 protein) and adherent junctions (VE-Cadherin and B-catenin/a-catenin linked to Vinculin).

Research Question and Hypothesis

Do exosomes play a role in the pathogenesis of *diabetic retinopathy*?

We predict that the endothelial cells treated with non-diabetic (control) exosomes, should have a higher resistance than those treated with exosomes in diabetic conditions.

Culture of Human Endothelial Cells and Pericytes



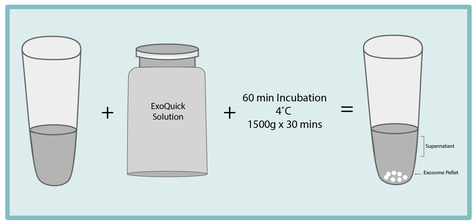
Human Retinal Pericyte Cells (4x)

Human Retinal Endothelial Cells (HREC) and Pericytes (HRPC) were cultured. Extracellular vesicles were isolated from the culture media of HRPC.

HREC were grown in Lonza EBM Endothelial Cell Growth Basal Medium and HRPC were grown in Cell Systems Complete Medium with Culture Boost and FBS.

HRPC were grown in two different conditions:
a.) 1M D-glucose + 20ng/ml TNFa + 20ng/ml IL-6 (Diabetes)
b.) 1M Mannitol (Normal)

Isolation of Extracellular Vesicles



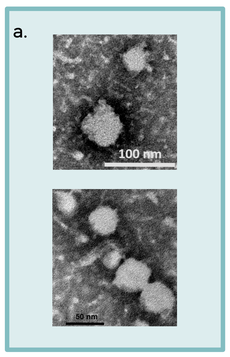
Extracellular vesicles were Isolated from the culture media of HRPC in diabetes and normal conditions.

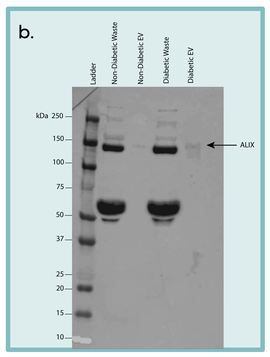
To isolate the EVs, we used a differential centrifugation method, ExoQuick-TC exosomes precipitation Kit.

Characterization of Isolated Exosomes

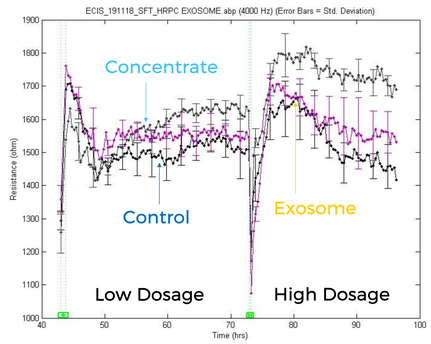
(a.) Western Blot analysis validated the correct protein presence of isolated *exosomes* using ALIX antibody.

(b.) Further characterization of successful *exosome* isolation was done using Transmission Electron Microscopy, showing correct exosome morphology and size (30-150 nm).



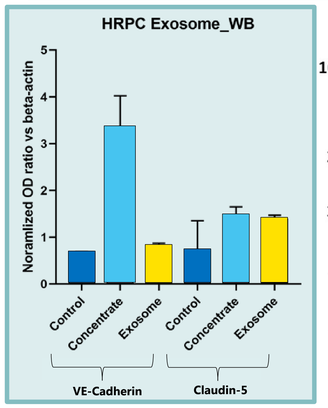


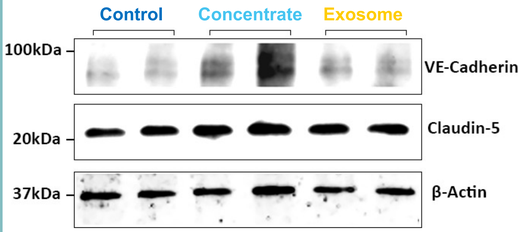
Results



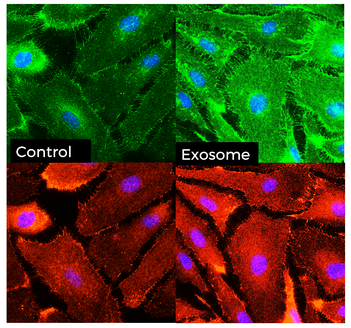
Human Retinal Endothelial Cells were grown in Electric Cell-Substrate Impendence Sensing (ECIS) arrays and were treated with control (medium), Concentrate , (supernatant) and exosome (purified/isolated exosomes) from normal conditions. Cells treated with exosomes showed a higher cell resistance than those treated with control. HREC treated with concentrate showed the highest cell resistance.

Results





The abundance of protein in our control, concentrate and exosomes were tested for VE-Cadherin and Claudin-5, and b-actin (loading control). Both concentrate and exosomes exhibited higher protein levels than control (medium).



HREC treated with control exhibited a weaker barrier structure shown by ZO-1 detection (green) and VE-Cadherin (red) compared to the cells treated with purified exosomes. The HREC treated with purified exosomes show a stronger expression of ZO-1 and VE-Cadherin, indicating a stronger cell barrier.

Summary of Results

Based on our data, it is suggested that EVs/exosomes play an important role in retinal vascular cell communications in normal physiology. The EVs/exosomes in normal, non-diabetic, conditions has a protective effect on the retinal endothelial cell's barrier structure and function.

Significance

- Further research is underway to identify the specific proteins involved in the endothelial barrier structure and function and the pathways that disrupt this barrier in diabetes. A better understanding of exosomes role in retinal vascular cell function could lead to potential clinical application to patients with DR.
- Future directions of our research consists of investigating what effects exosomes from diabetic conditions have on the cell barrier function and structure.

Current Research Question:

- Do exosomes from diabetic patients promote the pathogenesis of diabetic retinopathy?

Acknowledgements

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