Mitochondrial transplantation as a therapeutic strategy to attenuate diabetic endothelial dysfunction

Natalia Matiuto, BS[1], Bin Jiang, PhD[1,2]
[1]Department of Surgery, Feinberg School of Medicine, Chicago, IL, USA
[2]Department of Biomedical Engineering, Northwestern University, Evanston, IL, USA

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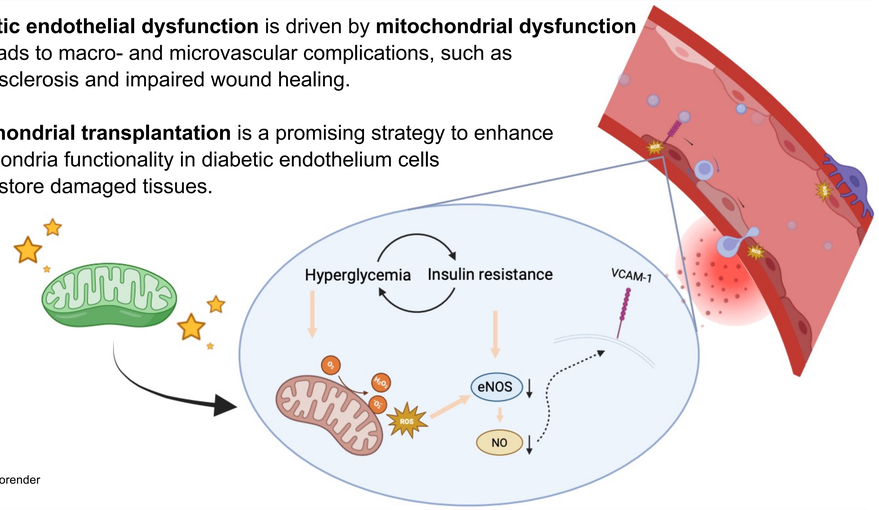
Introduction

- **Diabetic endothelial dysfunction** is driven by **mitochondrial dysfunction** and leads to macro- and microvascular complications, such as atherosclerosis and impaired wound healing.
- **Mitochondrial transplantation** is a promising strategy to enhance mitochondria functionality in diabetic endothelium cells and restore damaged tissues.



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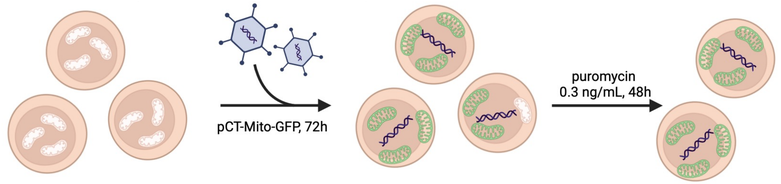
Hypothesis: Mitochondrial transplantation to diabetic endothelial cells restores endothelial function via alleviating mitochondrial dysfunction induced in diabetes.

Methods

1. Mitochondria Cyto-Tracer transduction optimization and selection of iPSC-MSC



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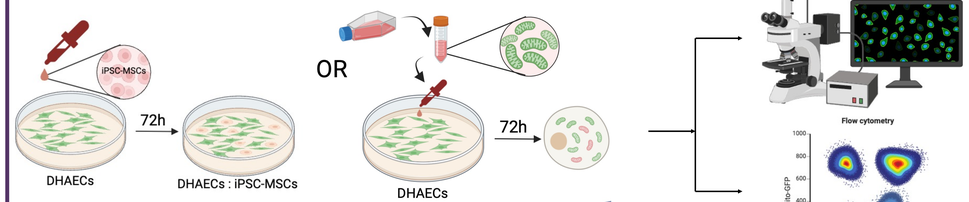
2. Coculturing iPSC-MSCs with DHAECs



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- Immunostaining was performed using CD-31 antibody
- Flow cytometry was performed using conjugated CD-31-APC antibody

3. Establishment of mitochondrial dysfunction in DHAECs

- DHAEC were conditioned in the EGM-2 supplemented according to the table for 5 days.
- Mitochondrial respiration, NO production, VCAM-1, ICAM-1, and P-selectin expression were assessed and compared to the control group.

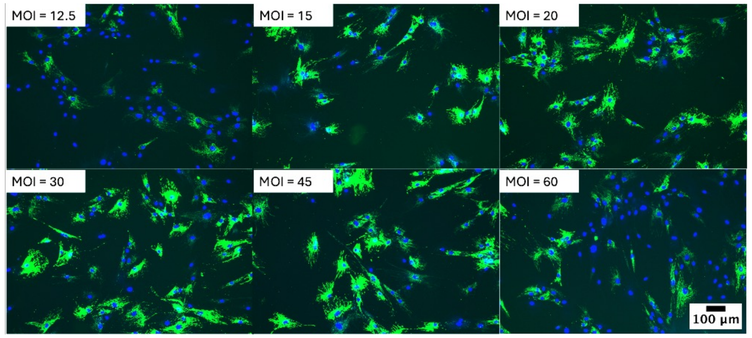
Supplemented EGM-2	Glucose	Urea	Cholesterol	TNFα
Diabetic conditions*	25 mM	9 mM	2.5 mM	20 pg/mL
Control (no changes)	-	-	-	-

*Supplement concentrations were used according to Gorashi et al, 2023[1]

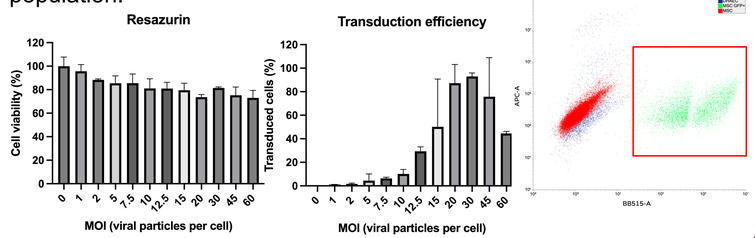
Results

Optimal MOI was identified as MOI=30 yielding in 93±2.96% transduction efficiency.

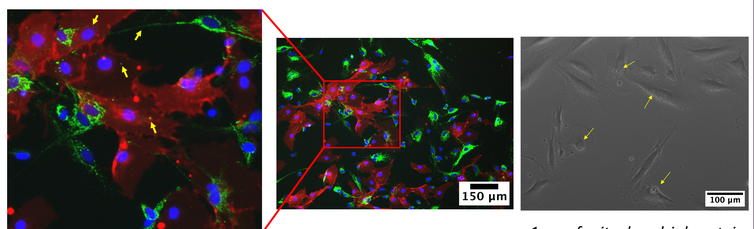
mitochondria | *nuclei*



Lentiviral transduction of MSCs with Mito-GFP and puromycin selection allowed obtaining consistently expressing Mito-GFP+ population.

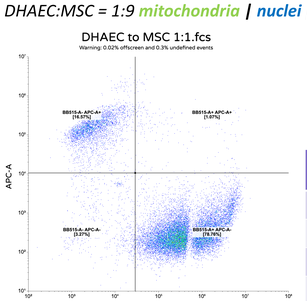


Fluorescence microscopy revealed successful transfer of mitochondria from iPSC-MSC in a cocultured system and autologous isolated mitochondria transplantation to DHAEC.



DHAEC:MSC = 1:9 mitochondria | nuclei | CD31

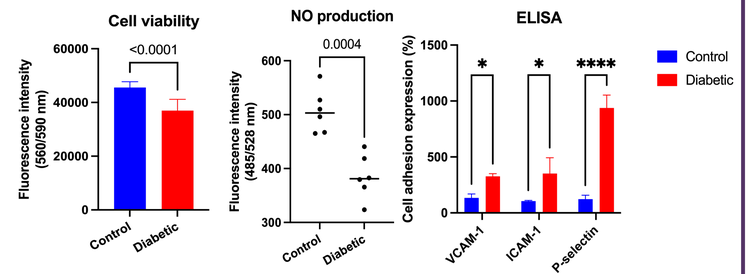
1 ug of mitochondrial protein per 5,000 DHAEC

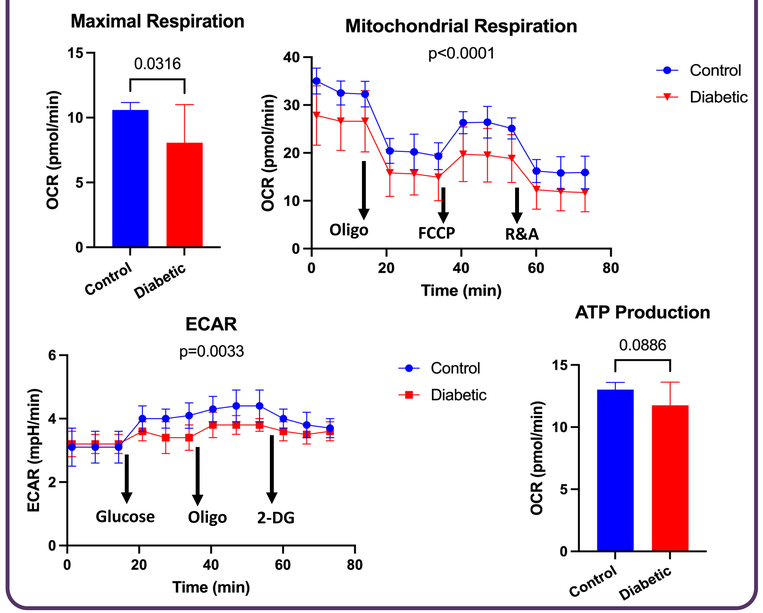


Flow cytometry demonstrated mitochondrial transfer efficiency up to 30.03% that increased with increasing ratio of MSCs to DHAECs.

DHAEC : iPSC-MSC ratio	% of GFP+ DHAEC
1 : 1	6.06%
1 : 2	18.15%
1 : 4	30.03%
1 : 9	26.69%

DHAECs stimulated with diabetes-like cell culture medium exhibit signs of endothelial and mitochondrial dysfunction.





Conclusions

- iPSC-MSC Mito-GFP transduction conditions were optimized at MOI=30.
- Mitochondrial transfer from iPSC-MSC to DHAEC and autologous mitochondria transplantation were validated and transfer conditions were optimized.
- Diabetic environment stimulation led to endothelial and mitochondrial dysfunction in DHAEC.
- Mitochondria fate in recipient cells, post-translation functional improvements, and exact mechanism underlining those will be studied in future work.

Acknowledgements

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References

1. Gorashi, Rayyan, et al. "Modeling diabetic endothelial dysfunction with patient-specific induced pluripotent stem cells." *Bioengineering & Translational Medicine* 8.6 (2023): e10592.