Engineering Mitochondria Delivery Systems to Regenerate the Vascular Endothelium

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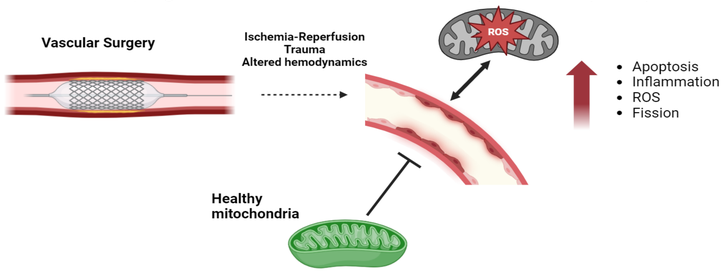
Introduction

Background: Mitochondria transplantation has emerged as a promising regenerative therapy for cardiovascular disease[1]. However, current transplantation strategies suffer from a lack of specificity, limited uptake, uncontrolled biodistribution, and overall subpar efficiency[2]. To overcome this, we are developing modular mitochondria delivery systems (MDS).



Hypothesis: Coating isolated mitochondria with collagen binding peptide (CBP) enables targeted delivery to damaged endothelium

Goal: Develop an endothelium-specific coating for targeted mitochondria transplantation for vascular injury

Methods

Mitochondria Isolation from Mesenchymal Stem Cells

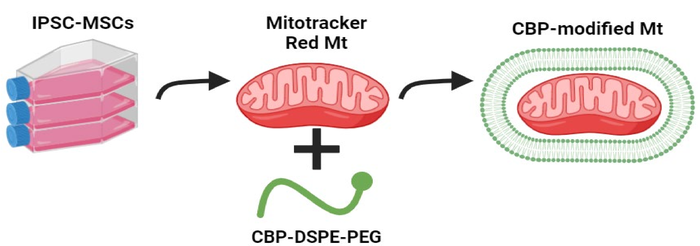
- Mesenchymal stem cells (MSCs) were differentiated from induced pluripotent stem cells (IPSCs) obtained from a young, healthy donor.
- Mitotracker Red-labelled mitochondria (Mt) were isolated using Mitochondria Isolation Kit for Cultured Cells (Thermo).

Peptide-Polymer Conjugate Synthesis

- **FITC-labelled CBP** was reacted with DSPE-PEG at a 1:1 thiol : maleimide ratio
- Reaction product was purified with dialysis+ characterization with NMR
- Collagen binding affinity was measured in vitro using collagen coated plates.

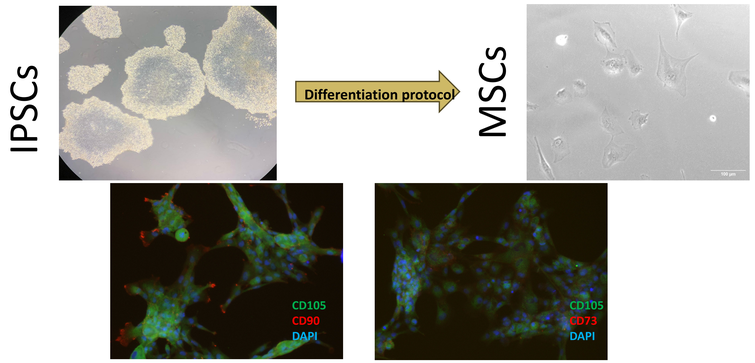
Mitochondria Coating

- Mt were incubated with CBP-DSPE-PEG conjugates at increasing ratios of polymer to mitochondria mass.
- Coating was visualized with confocal microscopy.
- Particle size distribution was measured with a Zetasizer.
- Mitchondria fucntion after coating was assessed using the Seahorse Mito Stress Test assay (Agilent).

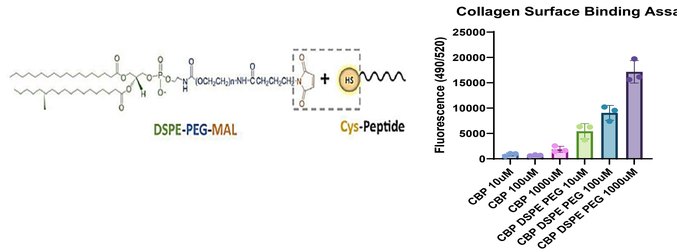


Results

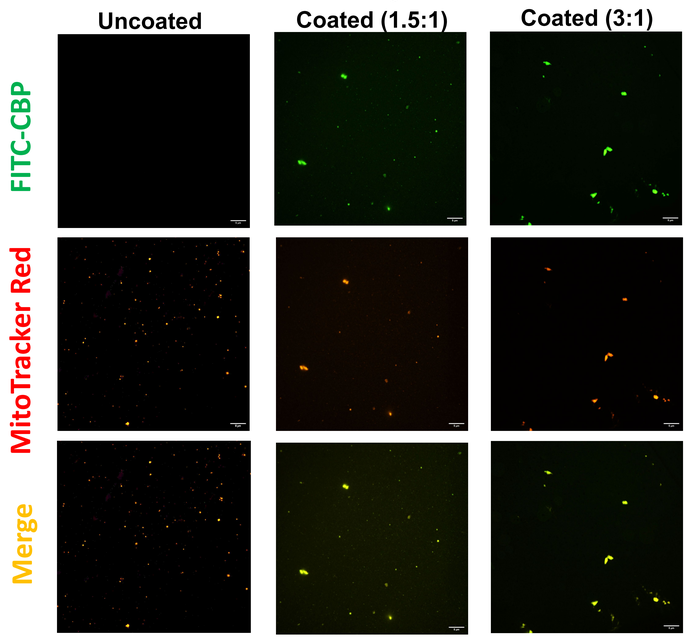
Successful Differentiation of MSCs from IPSCs



CBP-DSPE-PEG Retains Collagen Binding Affinity

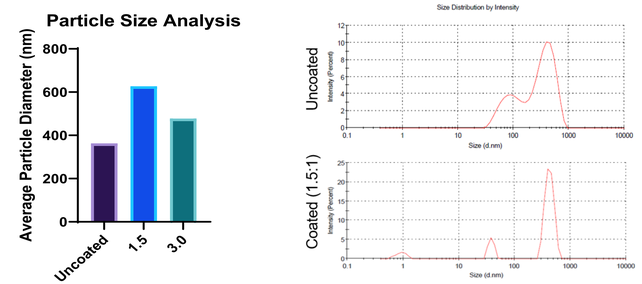


CBP-DSPE-PEG Effectively Coats Mitochondria

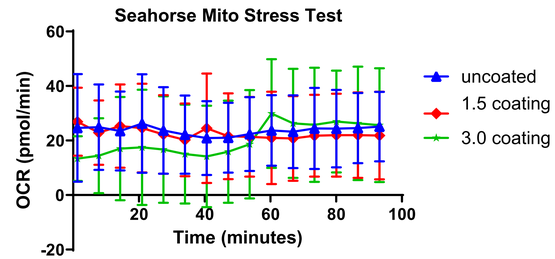


Results

CBP-DSPE-PEG Coating Alters Particle Size

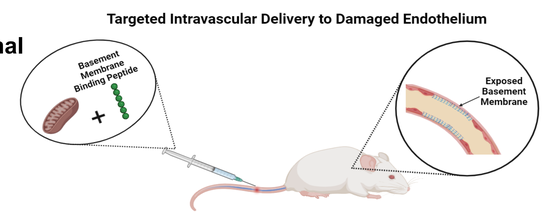


Polymer Coating Improves Mitochondria Respiration



Future Directions

- **Uptake and Functional Improvement with Endothelial Cells**
- **Rat Carotid Balloon Injury Model**



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References

1. Masuzawa, A., et al., *Transplantation of autologously derived mitochondria protects the heart from ischemia-reperfusion injury.* American Journal of Physiology-Heart and Circulatory Physiology, 2013. **304**(7): p. H966-H982.
2. Huang, T., T. Zhang, and J. Gao, *Targeted mitochondrial delivery: A therapeutic new era for disease treatment.* Journal of Controlled Release, 2022. **343**: p. 89-106.