Methodology for visualization of mitochondria in induced pluripotent stem cells and induced trophoblast stem cells

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Introduction

- The cause of preeclampsia (PE), a common and potentially fatal pregnancy syndrome, is unknown. However, early-onset preeclampsia (EOPE) has been associated with mitochondrial dysfunction.
- Our lab has generated induced Pluripotent Stem Cells (iPSCs) by reprogramming umbilical cord (uc) fibroblasts from both preeclamptic and healthy pregnancies.
- Additionally, the uc fibroblasts were converted to induced trophoblast stem cells (iTSCs), which represent the placental cells of early pregnancy.
- While iTSCs are already trophoblasts (TBs), iPSCs must be differentiated to TB using the BAP treatment method established by our lab
- We have established methodology for identifying mitochondria in iPSCs differentiated to TB and iTSCs using MitoTrackerRed, DAPI, and Phalloidin.
- Confocal microscopy was used to create three-dimensional images that can be analyzed further to compare the volume of mitochondria in control and EOPE TB or iTSCs and further support other mitochondrial analyses in our unique early pregnancy stem cell models.

Hypothesis

We hypothesize that if EOPE affects the mitochondrial quality but not quantity, there will not be a difference in signal when we quantify the amount of MitoTrackerRed.

Experimental Design

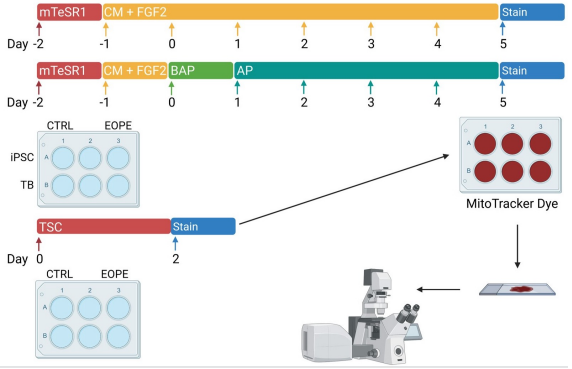


Figure 1. Experimental timeline.
The BAP treatment consists of bone morphogenetic protein 4 (BMP4) and signaling inhibitors A83-01 and PD173074, which inhibit ACTIVIN A and FGF2, respectively. The iTSCs are cultured for 48 hours. These experiments were performed at 5% O_2 (physiological) or 20% O_2 (oxidative stress). (*Milano-Foster*)

MitoTrackerRed Mechanism

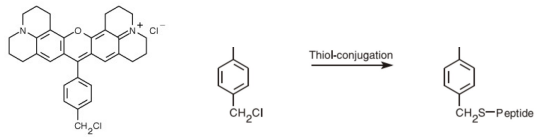


Figure 2. MitoTrackerRed Molecule
MitoTrackerRed reveals mitochondria by diffusing through the mitochondrial membrane of both whole and fragmented mitochondria. Thiol conjugation prevents rotation, and consequently fluoresces.

Staining Procedure

- Cells incubated with MitoTrackerRed Probe for 30 minutes
- Washed with PBS media
- Fixed with paraformaldehyde for 15 minutes at room temp
- Washed with PBS and stored in fridge
- To stain with Phalloidin, cells were washed with PBS
- Set with 0.1% Triton X-100 for 20 minutes
- Stained with Phalloidin stock
- Mounted coverslip on a microscope slide with DAPI

Results

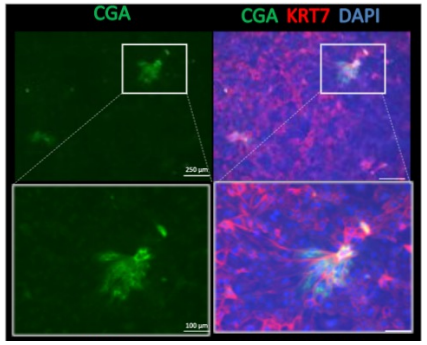


Figure 3. Immunolocalization of CGA and KRT7 in BAP differentiated iPSCs.
CGA is expressed in syncytiotrophoblast populations, indicating successful differentiation by BAP treatment. KRT7 is expressed by all TB populations. (*Milano-Foster, unpublished*)

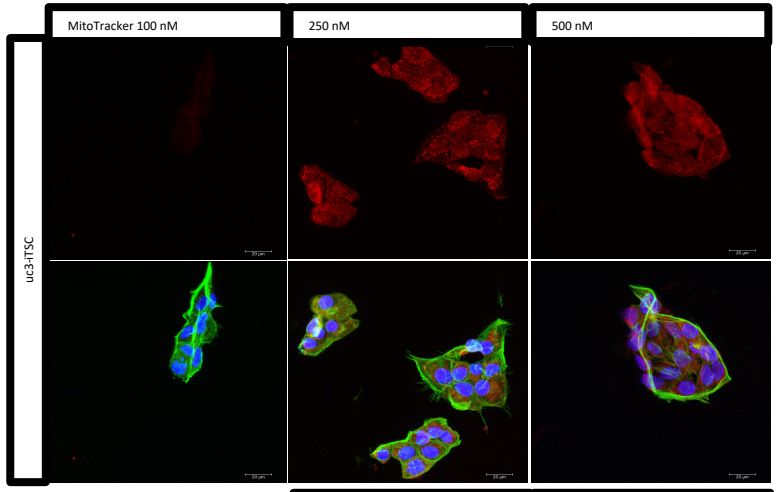


Figure 4. iTSCs stained with MitoTracker (Red), Phalloidin (Green), and DAPI (Blue).

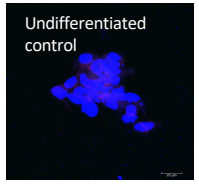
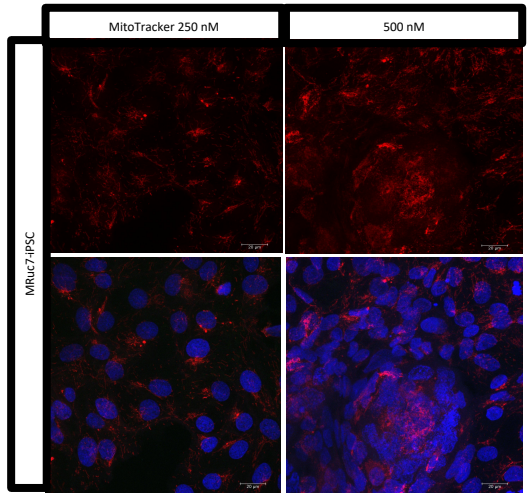
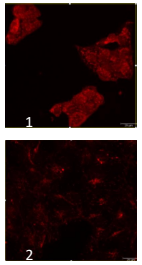


Figure 5. iPSCs stained with MitoTracker (Red) and DAPI (Blue). Undiff control did not receive BAP treatment.



Quantifying MitoTrackerRed Intensity



	Area	Mean
1	1346625	8.623
2	1341990	6.393

- Using Fiji software, we can compare the amount of MitoTrackerRed signal fluoresced per cell. A higher mean indicates more Mitochondria localized in the set area.
- Using a T-test we can determine our results are significant.

Figure 6. Mean Signal intensity of 250nM MitoTrackerRed for uc3-iTSC and MRuc7-iPSC

Summary

- Using a concentration of 250nM for the MitoTrackerRed gave the best staining results.
- We have established a reproducible MitoTrackerRed technique for iPSCs and iTSCs and a basic model for quantifying MitoTrackerRed with Fiji

Future Directions

- We can compare the mitochondria of the control vs EOPE, differentiated vs undifferentiated, and 5% O_2 vs 20% O_2 incubation.
- Doing MitoTrackerRed analysis can show that there is no difference in the amount of Mitochondria, but there are differences of the functionality of the mitochondria, leading to further investigation of hydrogen peroxide and superoxide production.

Acknowledgements

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