NADH Fluorescence Recovery after Photobleaching (NADH-FRAP) for *in-situ* assessment of cardiac TCA cycle enzyme activity.

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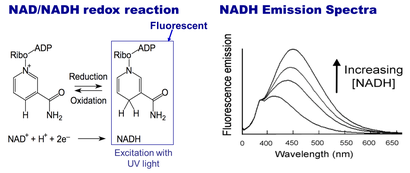
Background

- Enzymes act as biological catalysts and are involved in the regulation of many cellular processes.
- The reduced form of the coenzyme nicotinamide adenine dinucleotide (NADH) is studied for its key role in cell metabolism and its involvement in many redox reactions.



- NADH can be monitored by exciting tissues with ultraviolet (UV) light as NADH possesses endogenous fluorescence and changes in the amplitude of the signal indicate a change in the balance between NADH production and utilization.
- By photobleaching the NADH fluorophore, it is possible to use the rate of recovery to determine the rate of NADH production alone, and thus a metric of cellular metabolism and enzymatic activity [1]-[3]-[4].

Purpose

To expand NADH-FRAP to intact, whole hearts using new light-emitting diode (LED) technology and analyze its response depending on the photobleaching parameters and also on the conditions implemented.

Methodology

Animal model:

- Adult Sprague-Dawley rat hearts were quickly excised, cannulated via the aorta, and Langendorff perfused with oxygenated Tyrode's solution at a constant pressure and constant temperature .
- The hearts were mechanically arrested using a solution of 15mM of 2,3-butanedione monoxime (BDM) [5].
- A 3-electrode electrocardiogram (ECG) was used to record their electrophysiological response.

Imaging protocol:

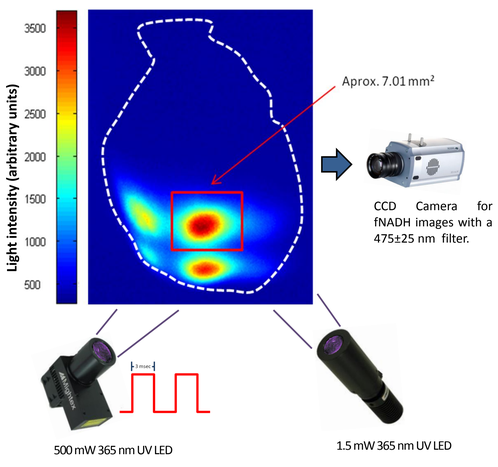
- Two 365nm UV LED sources were used to excite epicardium: one low power light (1.5mW) for continual imaging and one high power light (500mW) for bleaching focused on 4 different areas of approximately 7.01 mm^2 each.
- A CCD camera mounted with an emitted light filter of 475±25nm captured the NADH fluorescence signal.
- A custom LabVIEW program was used to control the lighting and acquisition of the signal: 5 secs of baseline, followed by a train of square pulses for the photobleaching phase, finishing with a recovery period.

Necrosis verification protocol:

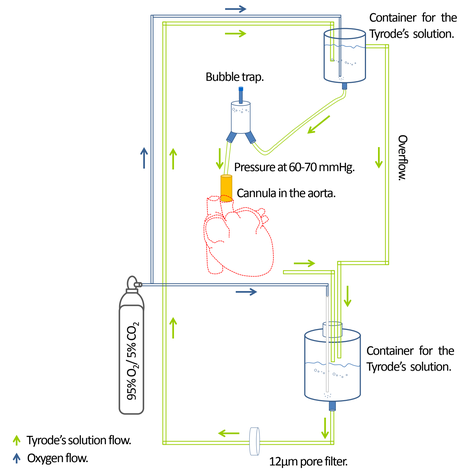
- Hearts were incubated in triphenyl tetrazolium chloride (TTC) dissolved in a phosphate buffered saline (1X PBS) solution at 37ºC during 10 minutes.

Experimental Setup

Imaging Setup



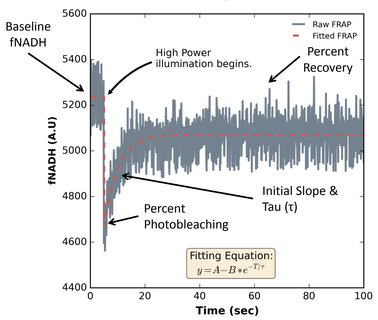
Langendorff Setup



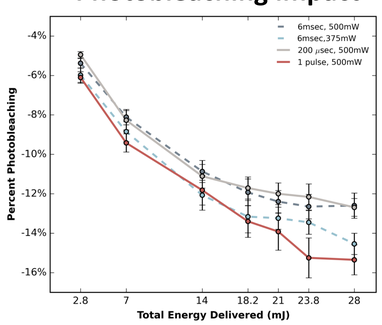
Results

NADH-FRAP parameters assessment

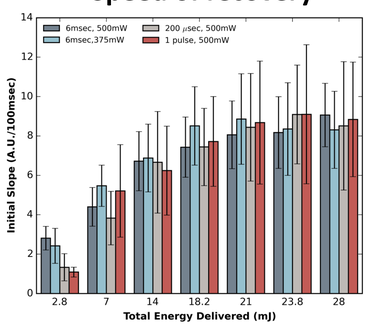
Extracting the info



Photobleaching impact

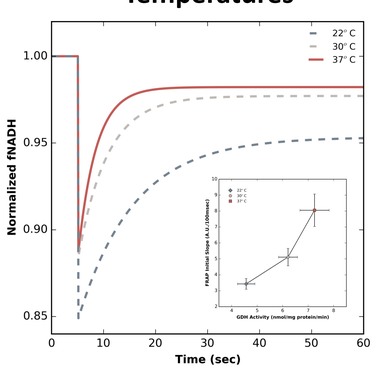


Speed of recovery

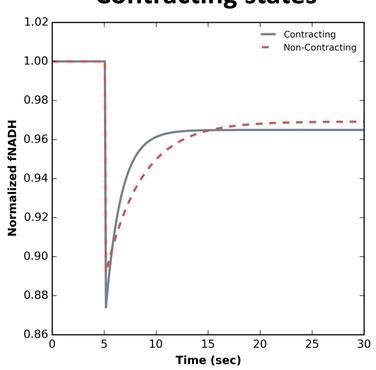


NADH-FRAP under different energy demand scenarios

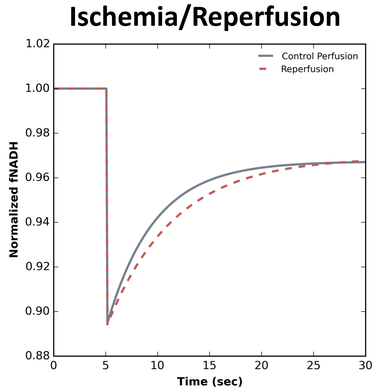
Temperatures



Contracting states

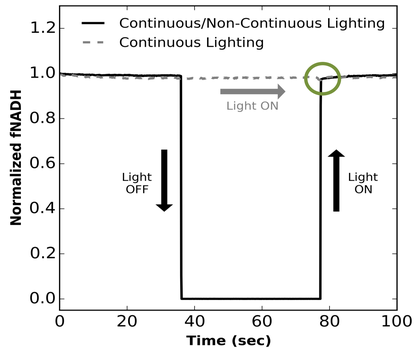


Ischemia/Reperfusion

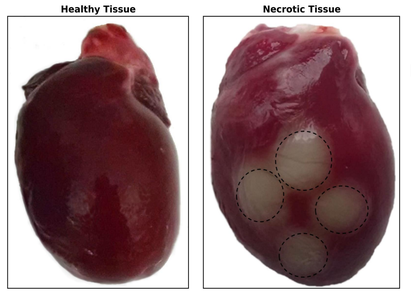


Results

Safety Verification



Low Power UV excitation LED ***does not causes photobleaching***.



(Left) **No tissue necrosis** is evident after a **standard NADH-FRAP** protocol. (Right) Positive control under abnormal conditions.

Conclusions

- Depth of photobleaching and recovery rate of NADH is directly proportional to the energy delivered.
- Faster recovery could mean more capacity and performance of NADH production.
- This FRAP technology could be potentially applied to other conditions, enzymes and organs as well.

References

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Acknowledgements

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