Verification of fine needle optical probe sensitivity to changes in NADH and FAD fluorescence

RESEARCH AND DEVELOPMENT CENTER BIOMEDICAL PHOTONICS

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Introduction

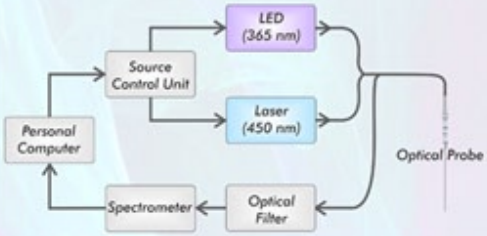
Fluorescence spectroscopy (FS) method widely finds its application in optical biopsy. Nicotinamide adenine dinucleotide (NADH) and flavin adenine dinucleotide (FAD) have descent fluorescence spectra sensitive to metabolic changes. However, a fluorescence spectrum also includes fluorescence of other fluorophores. It is essential to take into account the contribution of the coenzymes to the total recorded signal.

The Aim

To evaluate the sensitivity of fluorescence spectroscopy channel of optical biopsy device to the content of endogenous fluorophores associated with cellular metabolism.

Materials & Methods

Fluorescence channel of custom developed fine needle optical biopsy system:



- 365 nm source for **NADH** fluorescence excitation.
- 450 nm source for **FAD** fluorescence excitation.
- 400 and 495 nm longpass filters for attenuating backscattered radiation.
- 350–1000 nm CCD spectrometer for spectra recording.
- Fiber optic probe: fine needle with 1 mm outer diameter.
- Data processing: using custom-developed software in MATLAB.

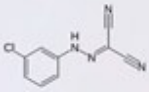
Animal model:

Wistar male rats

Investigated areas:

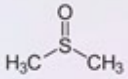
The studies were approved by the Ethics committee of Orel State University.

Solutions applied to the studied areas:



Carbonyl cyanide m-chlorophenylhydrazone (CCCP)
(0.01, 0.1, 1 mmol)

Increases the permeability of mitochondrial membrane, stimulates oxygen consumption.



Dimethyl sulfoxide (DMSO)
(1%, 10%, 100%)

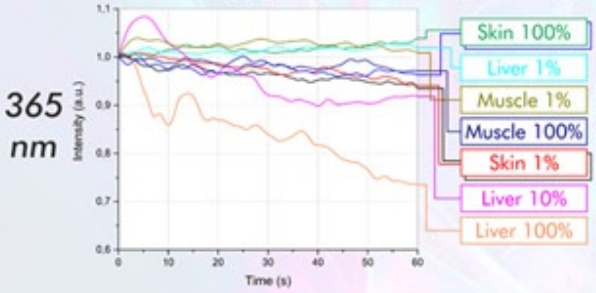
Organosulfur solvent, optical clearing agent.

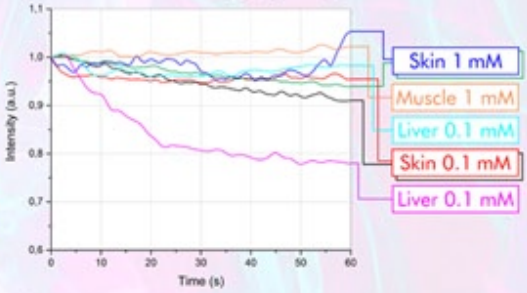
CCCP was diluted in DMSO. DMSO was diluted in phosphate buffered saline. Concentrations of CCCP and DMSO were variated to find optimal values for different types of tissues.

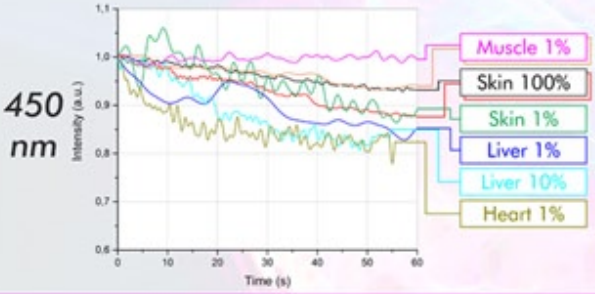
Results & Discussion

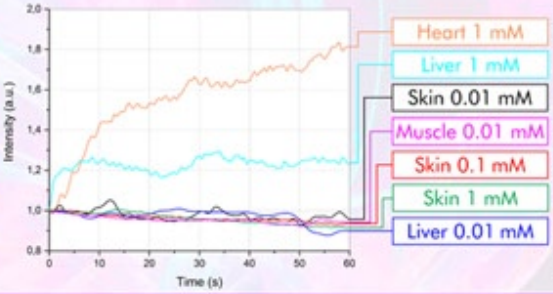
After applying DMSO (control)

After applying CCCP









- Significant influence of photobleaching was observed in most cases.
- After applying the CCCP solution 365 nm fluorescence decrease in some cases became higher, which can be associated with the effect of CCCP causing the oxidizing of NADH and fall of the fluorescence signal.

- Applying of CCCP solution caused a significant increase of fluorescence in the heart and liver tissues under 450 nm. In other cases, photobleaching was still observed, but its decrease became lower. CCCP causes increased levels of FAD, which leads to increasing of fluorescence signal.

Conclusion

- DMSO and CCCP have caused different influence in different organs. Effect of CCCP was seen more clearly in the liver and the skin, while the skin and the muscles were more resistant.
- The results showed the ability of developed channel to register the changes of fluorescence caused by the very changes in mitochondrial metabolism. The possibility to adapt the methodology of cell metabolism studies to whole organs was tested.
- Further studies seem promising to adjust the concentrations of DMSO and CCCP substances more precisely for certain organs. The results obtained will be for more accurate interpretation of fluorescence spectroscopy data.

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