Monitoring programmed cell death of living plant tissues in microfluidics using electrochemical and optical techniques

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Poster abstract

Monitoring programmed cell death of living plant tissues in microfluidics using electrochemical and optical techniques

Christina Mark[1,2], Kinga Zór[2], Arto Heiskanen[2], Birte Svensson[1], Jenny Emnéus[2], Martin Dufva[2] and Christine Finnie[1]

[1]Enzyme and Protein Chemistry, Department of Systems Biology, Technical University of Denmark, [2]Department of Micro- and Nanotechnology, Technical University of Denmark

Programmed cell death (PCD) in plants can influence the outcome of yield and quality of crops through its important role in seed germination and the defence process against pathogens. The main scope of the project is to apply microfluidic cell culture for the measurement of electrochemically or optically detectable events during PCD in barley aleurone layer, a cell model for living plant tissues, for a better understanding of the underlying mechanisms of PCD in plants.
Microfluidic cell culture enables *in vitro* experiments to approach *in vivo* conditions. The major advantage of electrochemical sensors and detection systems is that they can be miniaturized, multiplexed and automated without losing their performance making them suitable for integration with microfluidic devices[1,2]. Combining microfluidics with electrochemical and optical detection allows implementation of a wide range of assays for online, real-time, parallel analysis of important parameters such as redox activity (NADPH:NADP ratio), H_2O_2 concentration, oxygen consumption, extracellular pH, cell viability and release of target enzymes (α-amylase and limit dextrinase).
Probing the intracellular redox activity is of major importance, since it is known that reactive oxygen species, which are affected by changes in the redox activity of the cells[3], are involved in PCD in plants, but the relationship between and mechanisms behind ROS and PCD is only poorly understood in plant cells[4]. Recently, it has been shown, using optical detection, that the H_2O_2 concentration changes depending on phytohormone activation or inactivation of aleurone layer metabolism and subsequent PCD[3].

Currently, we are working on the optimization of an intracellular whole-cell redox activity (NADP:NADPH ratio) assay[5] to be able to detect possible changes of the cellular redox activity in barley aleurone layer. In our initial experiments using the electrochemical mediator-assisted assay we observed changes in the redox activity with tendencies similar to those for the H_2O_2 concentrations presented by Ishibashi *et al*. Further experiments are needed in order to improve reproducibility of the measurements and to find the optimal parameters suitable for its application in the microfluidic device.
Meanwhile, we successfully detected PCD induced by phytohormones in barley aleurone layer using a double-fluorescent probe-system also used by Fath *et al*[6], and it is planned to integrate this system in the microfluidic device.

References

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