PATCH-CLAMP ELECTROPHYSIOLOGICAL MEASUREMENTS ON SINGLE CELLS UNDER HYPOXIC CONDITIONS IN MICROFLUIDIC SYSTEMS

Ahmed Alrifaiy1,2, Olof A. Lindahl1,2,3, Kerstin Ramser1,2

1Luleå University of Technology, Luleå, Sweden. 2CMTF, Centre for Biomedical Engineering and Physics, Umeå, Sweden. 3Umeå University, Umeå, Sweden.

Abstract
We present a multifunctional system, a mini laboratory that fits on a microscope, for electrophysiological investigations on neurons with control over the milieu, e.g., biochemical and hypoxic conditions related to diseases such as stroke to understand the molecular defense mechanisms of the brain. A closed PMMA based microfluidic system was developed, in which a patch clamp micropipette was fixed in a specified position within the microfluidic channel. Biological cells were trapped optically and steered through the microchannel towards the tip of the pipette. Patch clamp electrophysiological measurements were acquired under hypoxic variations of cell environments. Experiments have shown sufficient control over the oxygen content within the microfluidic system (~1 %), and there was no remarkable photo-effect induced by the trapping laser on the investigated cells.

Keywords: microfluidic systems, patch-clamp, optical tweezers.

1. Introduction
Stroke includes brain damage caused by oxygen deficiency within the brain. A protein called Neurogobin, (Ngb) that is found within neurons in some parts of the brain has shown a protective role against hypoxia-related brain damage1. To understand the protein's function in living (in vivo) conditions, we developed a functional system to reproduce hypoxia on a chip. A multifunctional system was developed to study the response of functional individual neurons exposed to different chemical environments in real time. The system included the patch clamp technique1 for investigation of the electrophysiological activity of the neurons, a closed microfluidic system with control over the environment and optical tweezers2 for cell manipulation.

2. Methods and materials
The traditional way of patch clamp recordings is to move a pipette to a biological cell in an open system like a Petri dish. This concept is here replaced by a closed microfluidic chamber combined with optical tweezers for contact-free cell manipulation3. A PMMA closed microfluidic system was fabricated by CNC machining with a fixed micropipette. The system was fitted on an inverted microscope (IX71, 100x, 1.4 NA, Olympus, Japan). A multi-perfusion pump system (Cetoni, Gernany) was used for introduction of cells (Yeast, Saccharomyces cerevisiae) and buffers with different chemical properties and oxygen levels. The optical tweezers was build upon a NIR diode laser (200mW, 808nm, IQ1A, Power Technology, USA) and optical components (Thorlabs, Sweden). Patch clamp electrophysiological signals were recorded using a signal amplifier (Heka 7, Germany), Digidata1200 interface and software program pClamp7 (Axon, USA), see figure 1.

3. Result and discussion
Patch clamp measurements were carried out to record the electrophysiological responses of yeast cells within the close system under varying oxygen content. Yeast cells were trapped and manipulated optically through the microfluidic channels to the micropipette. The patched cells were exposed to hypoxic conditions using oxygen-free and oxygenated solutions, while electrophysiological measurements were performed. Experiments verified that the optical tweezers had no photo-effect on the investigated cells and the oxygen content within the microfluidic channels was about 1%.

4. Conclusion
The concept of combining microfluidic system, patch-clamp and optical techniques for single cell investigations under optimal control of the oxygen content has been established. Investigations are ongoing to improve the cell viability and transport within the lab-on-a-chip. The system will be refined to perform complete electrophysiological investigations with simultaneous monitoring of the biochemical composition and functioning of biological cells by optical spectroscopy.

References

Figure 1: The microfluidic system is fitted on the inverted microscope and linked to pump systems for insertion of solutions and cells. The integrated patch-clamp pipette is connected on a holder and a head-stage that are mounted onto a lab made stage and to a patch-clamp signal amplifier.