

Automated patch-clamping with the novel CytoPatch™ technology

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The knowledge about structure, function and regulation of ion channels and their involvement in various diseases has strongly increased. As a consequence, ion channels receive special attention as a target class for drug discovery by the pharmaceutical industry. To meet the needs of industrialized drug discovery adequate methods and instrumentations are required for screening a large number of compounds against this target class.

Ion channels, however are a target class hard to exploit, because reliable recording of ionic currents under a defined membrane voltage can only be obtained by the patch-clamp technique. However, this technique has not been available for screening due to its very low throughput. Devices for automated patch-clamping will overcome this limitation.

Patch-clamp automates for whole-cell recordings must (1) bring isolated cells in contact with patch contacts, (2) form gigaseals and (3) establish stable intracellular access that allows for high-quality recording of ionic currents. To settle this three tasks with a micromachined patch contact we developed the so-called CYTOCENTERING technique to apply to suspended cells the same operation sequence as in conventional patch-clamping. It combines the advantage of openings with diameter larger than 4 µm in planar substrates for attraction of suspended cells by moderate suction and pipette openings smaller than 2 µm for seal formation. With this method we immobilized selected cells from a flowing suspension on the tip of a patch pipette by suction with a success rate of 97 % and formed gigaseals with a success rate of 68 %. Subsequent whole-cell recordings and intracellular staining with Lucifer Yellow proved the stable access to the cytoplasm.

A micro-structured glass chip with embedded, concentric arranged suction and contact openings has been fabricated. This CYTOPATCH™ chip replaces the patch-pipette and all means for cell positioning. It allows to apply the same protocols for whole-cell analysis as the conventional whole-cell patch clamping does - with the same quality and information content.

The CYTOPATCH™ chip is a disposable that is reloaded automatically for each cell into the CYTOPATCH™ automat. For higher throughput (up to 10 000 cells per day, depending on the performed patch clamp protocol) the automat can operate multiple CYTOPATCH™ chips, individually and fully automated handled for asynchronous recording of up to 50 parallel-patched cells.