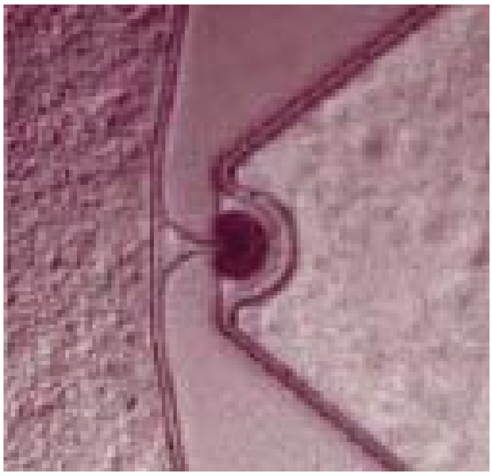
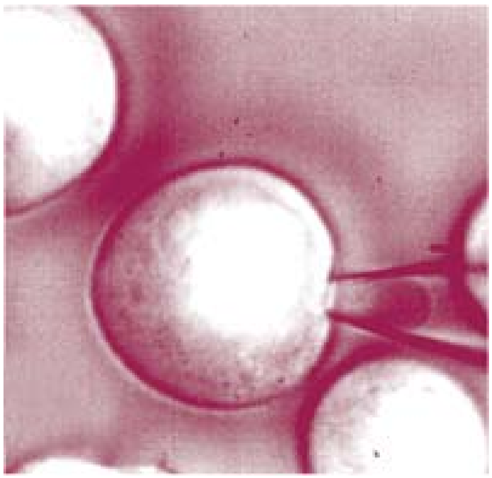
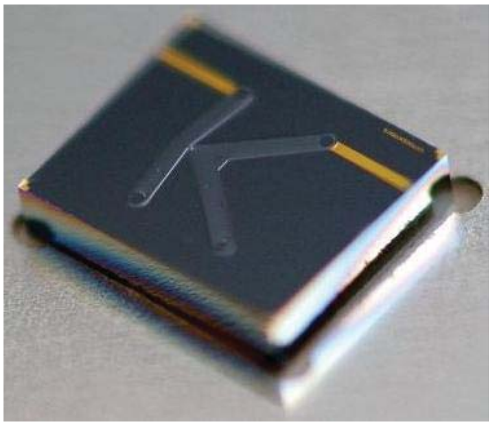
CytoPatch™2 Technology

The new standard in automated patch clamp technology







cytocentrics

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CytoPatch™ Technology

What is it? What does it do?

Ideally suited as a low / mid-throughput alternative to manual patch clamp

The CytoPatch™

... technology has been developed to produce manual patch clamp data quality from a fully automated system. The CytoPatch™ mimics the essential mechanics of the manual patch clamp technique and is an all-inclusive data acquisition, management and analysis system.
By combining hardware, software and proprietary microfluidic chip technology, the CytoPatch™ functions to produce manual patch clamp quality data from a fully automated, walk-away system. The microfluidic chip incorporating a true giga-seal pipette within a small perfusion "chamber" together with the unique continuous perfusion system with fast wash-in/out capability make the CytoPatch™ the perfect patch clamp platform for the study of both ligand- and voltage-gated ion channels in cell lines, stem cells and primary cell preparations.

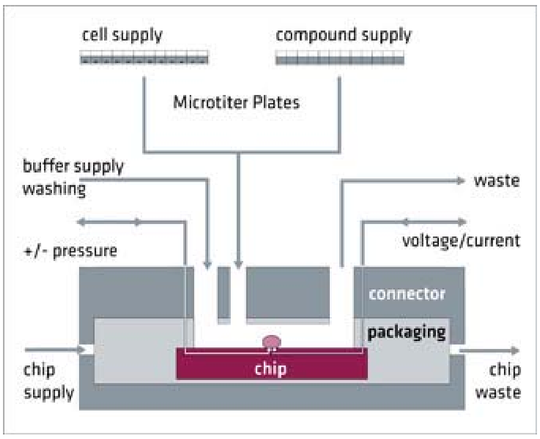
Guarantee of High Data Quality

Confidence in patch clamp electrophysiology depends on being able to make repeatable accurate measurements. Assurance of high data quality requires proper operation of the patch clamp circuitry in conjunction with careful assay design and analysis methods, each of which is a potential source of artifact or error introduction. The CytoPatch™ is the only automated patch clamp platform that generates data with the same high standard and level of trust as manual patch clamping does in the hands of experienced electrophysiologists.

The CytoPatch™ provides complete assurance of the same assay flexibility and high data quality as with manual patch clamp, and Cytocentrics guarantees attention to customer support and troubleshooting as an essential component of the integrated CytoPatch™ system.

The CytoPatch™ in Action: Overview

Each CytoPatch™ instrument is a fully equipped patch clamp system, with its own patch clamp amplifier and liquid handling system, and its own cell, buffer and compound supply. Multiple CytoPatch™ instruments can be networked together to create a virtual system for users requiring a higher throughput.

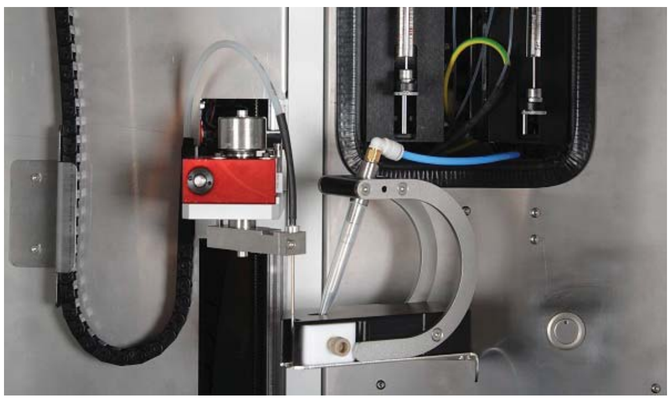


The graphic shows a representation of one CytoPatch™. The CytoPatch™ chip (shown in red) is placed on the Chip Holder Block within the CytoPatch™ Instrument. Needles puncture through the plastic packaging of the CytoPatch™ chip and fill in the necessary buffers into the microfluidic channels.
After filling the microfluidic channels, suspended single cells (provided in MTPs or cups) are injected into the CytoPatch™ chip. During measurements, online results will be created and displayed on the monitor of the workstation.

After a run, the Chip Holder Block moves down, disconnects the CytoPatch™ chip and drops it into a waste container. Waste fluids are collected in waste bottles.

To continue assay execution, the chip picker takes a new CytoPatch™ chip out of the chip tray – a new run starts.

Major Advantages of the CytoPatch™ Technology



Fully Automated Patch Clamp System

- Unattended operation, with walk-away times of up to 4 hours
- Easy operation; can be operated by laboratory technicians/assistants

Flexible Assay Design

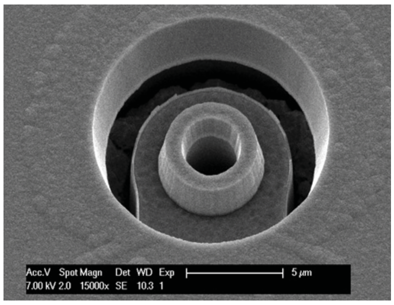
- Works with cell lines, stem cells and primary cell preparations
- Whole-cell, cell attached, and perforated patch protocols
- Works equally well with voltage gated and ligand gated ion channels
- Voltage and Current Clamp functionality

High Quality, High Information Content Data

- The microfluidic CytoPatch™ chips and sophisticated liquid handling system allow the system to closely mimic manual patch clamp processes and generate data of the same quality and information content as experienced electrophysiologists working on manual rigs.
- With the CPC2 chips, only 2 cells remain in the perfusion chamber during recording giving unmatched accuracy of pharmacological data

CytoPatch™ Technology

A Quartz-microstructured Patch Clamp Chip allows True Gigaseals

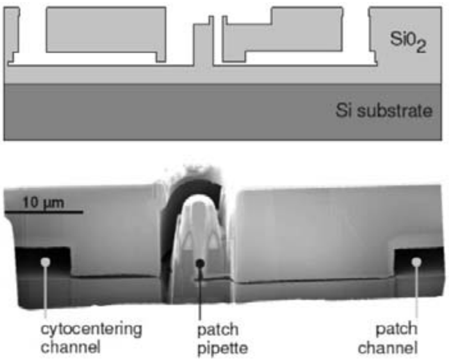


The heart of the CytoPatch™ Instrument is the micro-fabricated and patented CytoPatch™ Chip. The unique design of the CytoPatch™ Chip includes a glass pipette which mimics the properties of the conventional patch clamp electrode. The combination of two access channels, however, is unique compared to all automated patch clamp techniques and allows robust gigaseal recordings lasting over 30 minutes coupled with greater and superior perfusion control.

Unlike other automated voltage clamp platforms, the CytoPatch™ Instrument requires neither high Ca2+ buffer to support the sealing process nor fluoride in the intracellular solution to obtain gigaseals and stable whole cell recordings.

A sophisticated integrated microfluidic system allows the execution of defined and precisely triggered perfusion protocols.

With the special CytoPatch™ Chip design the conventional patch clamp process is mimicked, resulting in the same outstanding high data quality.



The most important prerequisite for good data quality is a high seal resistance above one GigaOhm. It is well known from manual patch clamp, that the patch pipette must kept clean of proteins or cell debris to obtain the high seal resistance. To accomplish this, typically a positive pressure is applied on the glass pipette, resulting in an outflow of clean intracellular solution.

This smart technique has been transferred to the CytoPatch™ Instrument: The glass pipette inside the CytoPatch™ Chip is surrounded by a second opening, the Cytocentering channel. This channel has the unique function to catch one cell out of the cell suspension: By the application of negative pressure it positions a cell onto the patch pipette before the sealing process starts.

During the particular process step of cell positioning, a simultaneously outflow of intracellular solution is generated in the patch pipette of the CytoPatch™ Chip. This is achived via the application of a positive pressure. The patch pipette of the CytoPatch™ Chip is kept clean and the seal performance is comparable to that of the manual patch clamp technique.

The CytoPatch™ Pipette resembles a „real“ patch pipette:

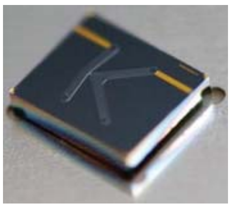
- Quartz glass for gigaseal formation
- Pipette shape and diameter comparable to MPC (2 µm)
- Cell Catch via Cytocentering Technology
- CytoPatchTM pipette is kept clean by positive pressure
- R_{pip} is 8 MW (Type Standard) or 4 MW (Type Enhanced)
- Unique in APC: Permanent perfusion

Benefit:

- High data quality due to stable gigaseals
- Robust whole-cell recordings

Working microfluidic unit

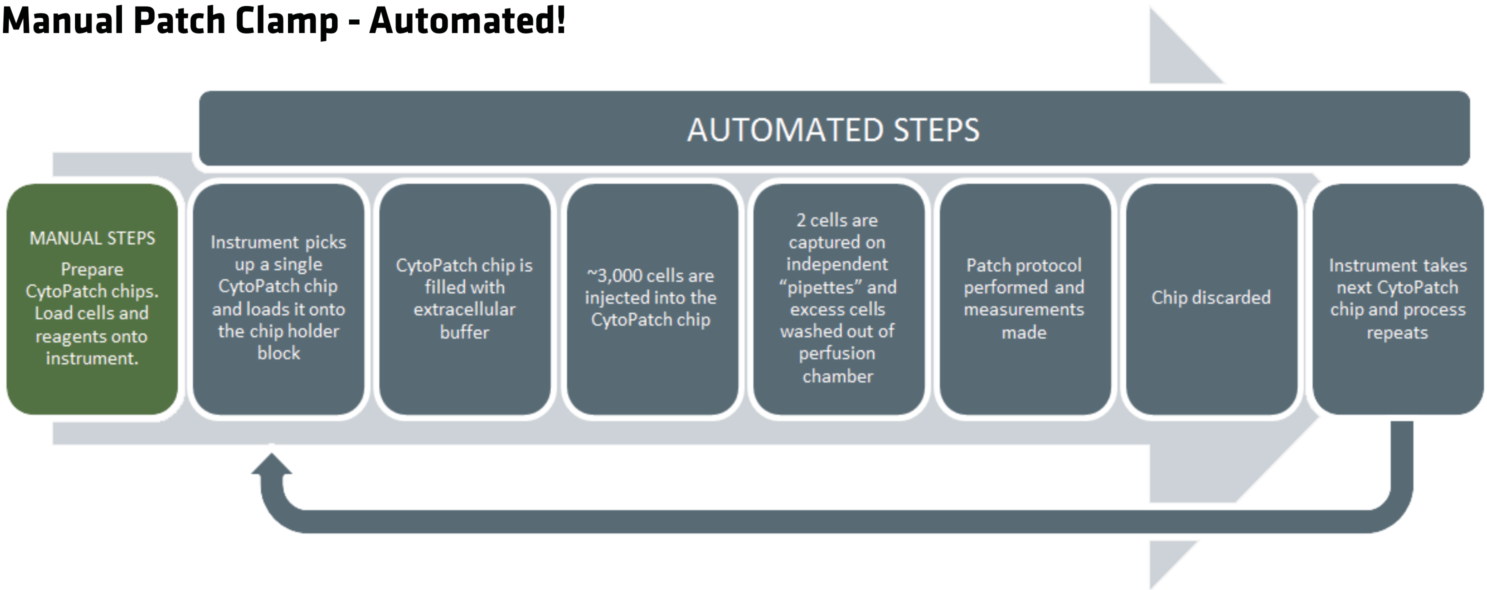
The unique "technology" generates high quality by closely mimicing the manual patch clamp process. The Cytopatch™ Chip & the Top Cap ("Packaging") combine to create the working microfluidic unit.



	Manual Patch Clamp Technique	CytoPatch™ Technique
Cell Capture	A common experience is that only clean patch pipettes result in true giga seal formation. To obtain this in conventional patch clamp a positive pressure is applied to the patch pipette. The intracellular solution flowing out keeps the pipette clean until the tip of the pipette is positioned close to the cell.	Suspended cells are injected into the chip close to the CytoPatch™ opening by a dispenser needle. A positive pressure is applied to the CytoPatch™ opening to generate an outflow of intracellular solution and to keep the patch pipette clean. A negative pressure is applied to the Cytocentering Channel to capture and position a single cell on top of the patch pipette
Sealing	A negative pressure in the patch pipette starts the sealing process.	Applying negative pressure in the CytoPatch™ opening starts the sealing process. In the Cytocentering opening the negative pressure is reduced.
Whole cell	The whole cell configuration is established by application of negative pressure pulses to rupture the membrane patch.	Similar to the manual Patch Clamp Technique
Record-ing	Ongoing perfusion during recording: With extracellular solution during control phase and washout, with compound during drug-application.	Similar to the manual Patch Clamp Technique

CytoPatch2™ Instrument

Manual Patch Clamp - Automated!



The CytoPatch2 instrument combines the assay flexibility and high data quality users expect from manual patch clamp with complete assay automation.

The CytoPatch2 has been designed to mimic the fundamental mechanics of the manual patch clamp technique, and provides the highest degree of accuracy of patch clamp data available from an automated platform, producing data of the same quality that experienced electrophysiologists generate from their manual patch clamp setups.

- Fully automated, walk away, patch clamp system
- Data quality equivalent to manual patch clamp systems
- Voltage and current clamp recordings
- Whole-cell, cell attached and perforated patch protocols
- Works with cell lines, stem cells, and primary cells
- GΩ seal resistances with physiologically relevant buffers
  - No need for seal enhancing buffers that can effect data quality
- Flexible perfusion system
  - Continuous compound perfusion ideal for “slow on” voltage gated ion channels
  - Ultra-fast compound wash-in (<5ms) perfect for fast desensitising ligand gated ion channels
- Only two cells in the perfusion chamber during recording
  - No drug sinks
  - Minimal compound usage
  - Unmatched accuracy of pharmacological data
- All new system housing with integrated temperature control
- Comprehensive “in-assay” biophysical quality checks ensure only the highest quality data is generated
- Scalable throughput
- GLP compliant

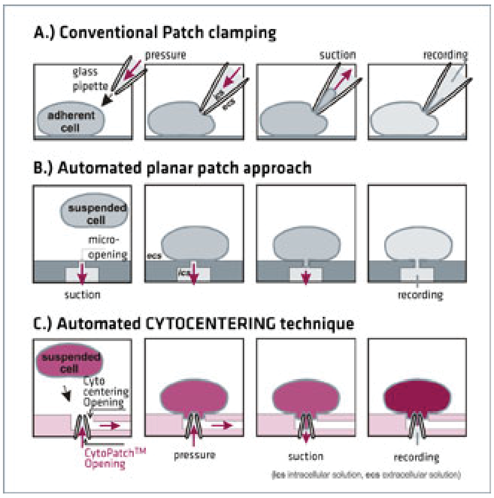
Each CytoPatch2 instrument has its own integrated patch clamp amplifier, liquid handling system, cell reservoir, and buffer and reagents supply modules. All process steps are executed consecutively without time delays as only a single chip is processed at a time.

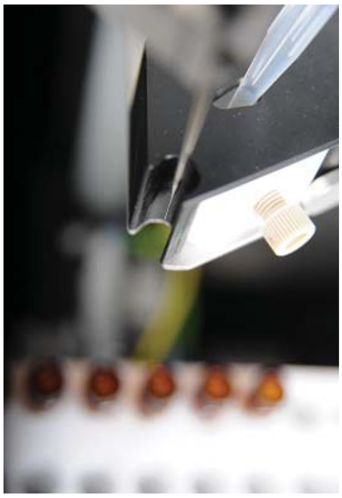
For customers requiring higher throughputs, the CytoPatch2 has been designed not only to operate as a standalone unit but also as part of a networked array of units. A CytoPatch™ network can consist of up to 20 individual CytoPatch2 units, all of which have their own PC’s and instrument software (The Assay Designer) so they can be operated independently and are networked to a server PC running The WorkflowManager software which controls the instruments as a group or multiple subgroups.

Single CytoPatch2 units are also perfect for assay development. Using the Cockpit software, users can now interact with the system in real-time and make “on-the-fly” adjustments to the protocols – just as you would with a manual patch clamp rig.

Comparison of CytoPatch2 with Manual Patch Clamp Technologies

Cytocentering Technology – A Technological Revolution in Patch Clamping





Comparision

A) Conventional Patch Clamp

The tip of a glass pipette is manually brought into contact with the membrane of an adherent cell. After application of a well-defined sequence of steps, the membrane patch under the tip of the pipette is ruptured. This enables intracellular access for clamping the membrane voltage and detecting the current flowing through ion channels.

B) Automated Planar Patch Clamp

Almost all automated patch clamp systems use what is known as “planar” patch clamp consumables in which the patch pipette is replaced by a hole in the bottom of a well through which suction is applied to capture a cell and ionic connectivity is established.

C) Automated Cytocentering™ Technique

Unlike any other automated patch clamp system, the CytoPatch2 uses the CPC2 chip in which two independent concentric channels are used per cell: The outer, Cytocentering, Channel has suction applied through it and is responsible for the positioning of the captured cell and the inner, CytoPatch™, Channel is used for patch clamp and recording.

Function

Seal & Patch Process At A Glass Tip Analogous To MPC

The Cytocentering Channel:

- Works equally well for wide variety of cell types, e.g. HEK, myocytes, neurons
- Auto-focuses cell onto seal aperture
- Only uses physiologically relevant buffers
- Creates gigaseals without the need for seal enhancers
- Low cell number per measurement (~1,500 per seal on the CPC2 chip)
- Only 2 cells retained in chamber during recording, therefore, no risk of drug sinks

In Combination With The CytoPatch2 Perfusion System:

- Unmatched accuracy of pharmacological data
- Continuous perfusion with 2 pumps
- Extremely fast solution exchange (4 ms for nAChR)
- No waste limitation

Advantages

Advantages :

The design of the CPC2 chip allows the CytoPatch2 to automate patch clamping by using a process analogous to cell capture on other planar automation system (cell attraction to an aperture by the application of suction) but allows the system to retain advantageous characteristics of manual patch clamp because of the physical similarities between manual glass pipettes and the tip of the CytoPatch™ Channel in the chip.

The separation of cell capture (by suction) and patch clamping in the CPC2 chip is favourable to a single channel planar solution, because the CytoPatch™ Channel is kept clean during cell capture by the outflow of buffer through the CytoPatch™ Channel enabling a high rate of gigaseal formation.

The flow-through design of the nanoliter scale perfusion chamber means that the system can function with only a small number of cells, which is increasingly important for researchers wanting to work with primary or stem cells. It also means that any cells not captured are washed out of the chamber before compound addition, reducing the amount of compound required and allowing pharmacologically accurate data to be produced from a known number of cells (either one or two).

CytoPatch2 – Offers a Cost-Effective Way of Bringing Fully Automated Whole Cell Patch Clamp Into Your Laboratory

Costs and Benefits

Investment cost for a CytoPatch™ are around 60% of a MPC work station.

Price Advantage

Variable cost per DP of the CytoPatch™ are around 20% of MPC work station.

Measureing Performance

Modularity of the CytoPatch™ technology increases the flexibility for the customers drastically and therefore allows them to meet any fluctuations in demand.

Comparison of Technologies – Graphical Overview

CytoPatch2 – Simplifies and Standardises Manual Patch Clamp

Manual Patch Clamp

problem

- Hand-pulled glass pipettes with variable diameter
- large bath chamber (vlume of up to 0.5ml)
- Home-made buffer systems
- Complex system hardware
- Complex amplifier software
- No standardization of measurements
- Difficult technique, highly skilled operators
- Labour intensive operation

CytoPatch™

solution

- CytoPatch™ Chips
- Miniaturized perfusion chamber (volume of 10nl)
- Cytocentrics optimized buffer systems
- Robust CytoPatch™ technology
- User-Friendly study set-up and evaluation software
- 100% standardized measurements
- Easy to use system, minimal training requirements
- Walk-away operation

Manual Perfusion System

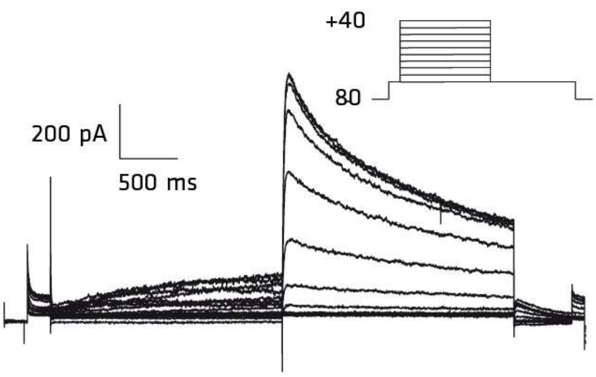
disadvantage

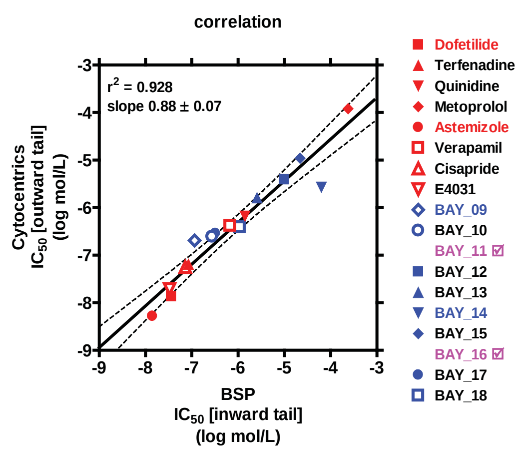
- High compound consumtion (10ml)
- Tubing change after compound application
- Special perfusion system required for ligand-gated ion channel recording
- Variable results with “sticky” compounds
- Complex assay development

CytoPatch™ Perfusion System

advantage

- Low compound consumtion (< 1ml)
- No tubing to change
- Excellent results with fast desensitizing lagand-gated ion channels
- Accurate results with “sticky” compounds
- Customized assay protocols delivered



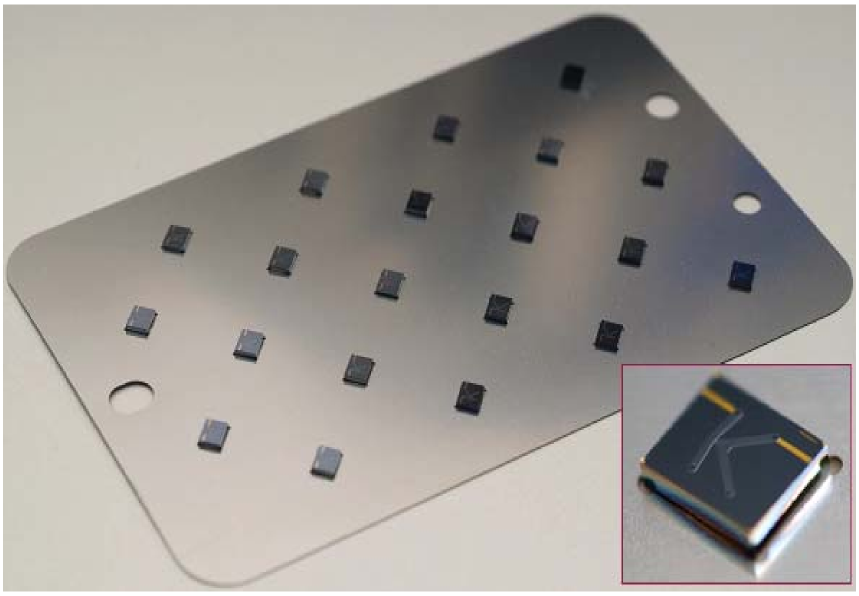


CytoPatch2 – Offers Advantages Compared to Both Manual Patch Clamp and Other Automated Patch Clamp Systems

APC (Parallel)	CytoPatch™	APC (Parallel)
Lower Data Quality	**High Data Quality**	**High Data Quality**
o Population Path	+++ Single Cell Measurements	+++ Single Cell Measurements
- No Giga seals without using seal enhancers	+++ Giga Seals	+++ Giga Seals
++ User independent	++ User independent	- User dependent
++ Lab technicians	++ Lab technicians	- Highly skilled electrophysiologist
+ Mass produced planar plastic connumables	++ Robust CytoPatch chips with glass patch pipettes	- Pipette variability (hand pulled)
++ QC'd buffers	++ QC'd buffers	o Home made buffer variability
0 Cell culture variability	++ Instant Cells Technology	o Cell culture variability
High Throughput	**Scalable Throughput (Low-Mid)**	**Low Throughput**
+ Multiple units / user	+ 20 CytoPatch™ unit / user	- One MPC unit / user
+ > 100 data points/ day / unit	o ~25 data point / 8h-day / CP unit	o ~20 data points / day / MPC unit
++ High CAPEX, high consumable cost, little upgrade path	++ User independent	- No throughput upgrade path
Limited Assay Flexibility	**Flexible Assay Design**	**Highly Flexible Assay Design**
o System Specific	+++ Voltage and current clamp	+++ Voltage and current clamp
o Different compound application limitations	+++ Continous perfusion & very fast application (<4ms)	o Different compound application limitations
- Many limitations on patch techniquie	o Some limitations on patch technique	+++ "Special" patch technique
- No manual control of experiment	+++ Manual control of experiment	+++ Manual control of experiment
+++ Automated operation	+++ Automated operation	- No automated operations
- Few upgrade options	o Some upgrade options	+ "Bolt-On" upgrade features

CytoPatch™ Microchip – Increased throughput for Patch Clamp

Designed for real gigaseal formation and reproducible data generation



The Cytocentrics microchip has a three-dimensional structure with a concentric double pipette of 2 and 13 microns in diameter. The double pipette has lateral channel connections. The chip top layer is made out of quartz glass and is designed to pass submicroliter fluids through quartz chip channels. This particular chip is manufactured with the 6 inch silicon wafer process. The chip was designed to position and contact single cells.

Conventional patch pipettes are not suitable for the automation of patch clamp. Therefore we have developed the CytoPatch™ Chip, which consists of quartz glass (SiO_2).

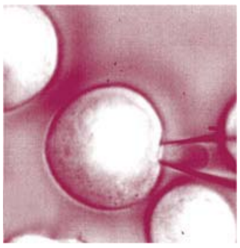
Quartz glass has outstanding electrical characteristics:

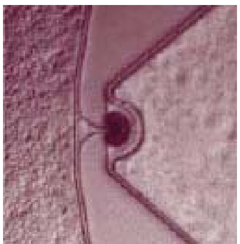
- Low dielectric constant
- Low noise level
- Excellent insulating material

Function

Two channels are embedded in the microstructured CytoPatch™ Chip: the Cytocentering Channel, which is for suction and positioning of the cell, and the CytoPatch™ Channel, which is for patch clamp and recording. The separation is an advantage over a one channel solution. The CytoPatch™ Channel stays clean and allows a high rate of gigaseal formation. Furthermore, the chip is optimal for fast replacements, resulting in high throughput rates.

This Cytocentering technique maps the conventional patch clamp procedure in an inverse configuration, applying the same hydro-dynamical and mechanical forces on the cell and the membrane as in the proven manual technique.





Chip Properties

- Single cell capture, membrane rupture and ion channel measurement
- Microchip with three-dimensional structure with concentric double pipette of 2 and 13 µm in diameter
- Double pipette with lateral channel connections
- Chip top layer made of quartz glass
- Designed to pass sub-microliter volumes of fluids through quartz chip channels

Manufacturing

The disposable CytoPatch™ Chip is manufactured by Cytocentrics BV in the cleanrooms of the Philips High Tech Campus, Innovation Center Eindhoven (The Netherlands). Our engineers have longstanding expertise in the development and production of microsystems technologies.

Sophisticated Software – Intuitive and Efficient

Leaves Nothing to be Desired: Tailored to Customers Needs

As the developer of all system components, we have many years of successful experience in operating the CytoPatch™ Instrument in our lab as a customer screening service. We are aware that the software must be easy to use and flexible enough to fulfil many and varied r equirements in order to facilitate your daily work.

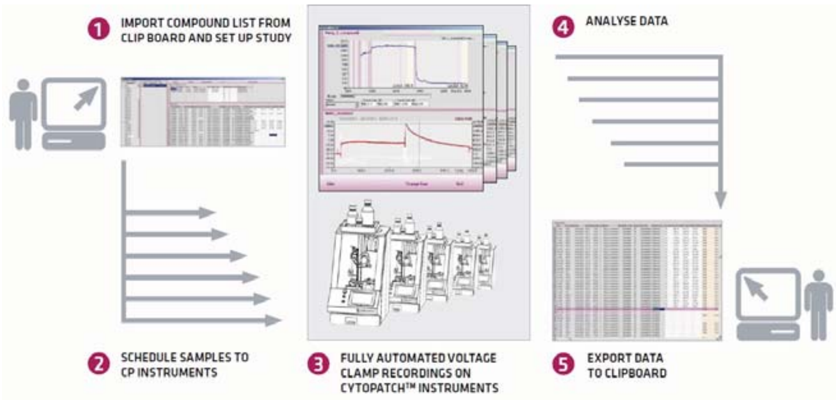
The Cockpit is a real-time interface with your CytoPatch system allowing you to use it just as you would a manual rig. Perfect for assay development.

The Assay Designer gives you drag and drop functionality to quickly create new protocols.

The Workflow Manager allows you to setup up new multi-compound pharmacological studies and schedule work over multiple CytoPatch workstations.

The CytoPatch™ Software (CPS) guarantee an efficient workflow and will meet your expectations:

- Intuitive to use Windows-based software
- Suited for GLP-conform studies
- Easy new study set-up with compound import function
- At a glance overview of study status and open data points
- Smart scheduling of compounds to the CytoPatch™ Instruments
- Real-time monitoring of recording results
- Comprehensive analysis tools
- Export options to further software programs



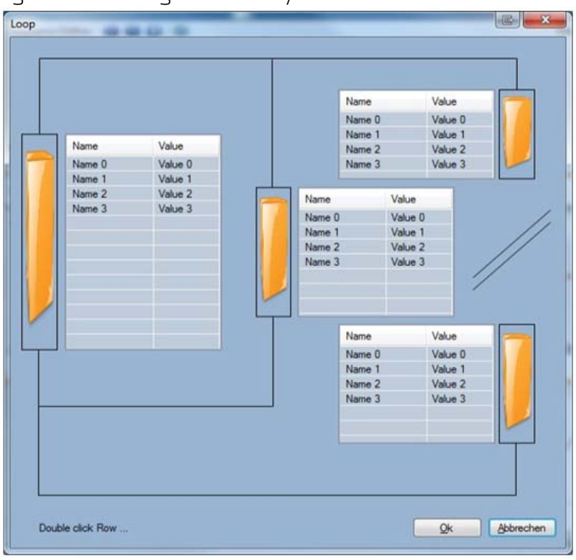
The CytoPatch™ Cockpit

for Academic Research

The CytoPatch™-Cockpit 2012 delivers full automation with a total manual control mode offering MPC assay flexibility. With its intuitive user interface the CytoPatch™ provides Scientists with the flexibility of MPC whilst giving the advantages of a fully automated Screening-Device.

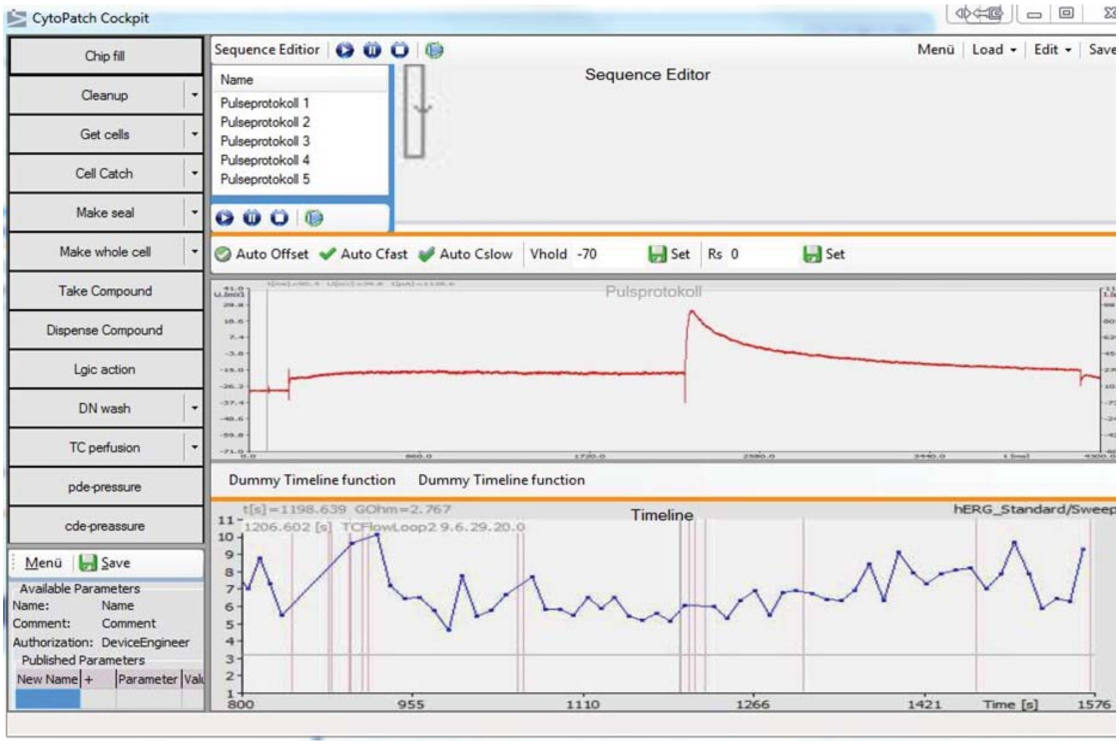
He comes with pre-defined options but offers access to all operational elements the Scientist needs to direct the experiment.

The CytoPatch™-Cockpit 2012 gives the same flexibility as MPC for experiment design and setup but provides a fully automated system on which to run them.



All New Software Interfaces:

- Easy to navigate user interface with direct access to main assay processes
- Pre-defined processes for fast operation
- Direct manual control assures full availabilty of experimental flexibility to Scientist
- Real time recording of all parameters
- Online monitoring of recordings and quality parameters



- Pulse protocol sequence editor enables flexible protocol design

- Online pulse protocol monitoring & recording
- Quick Buttons for amplifier s etting control and adjustment

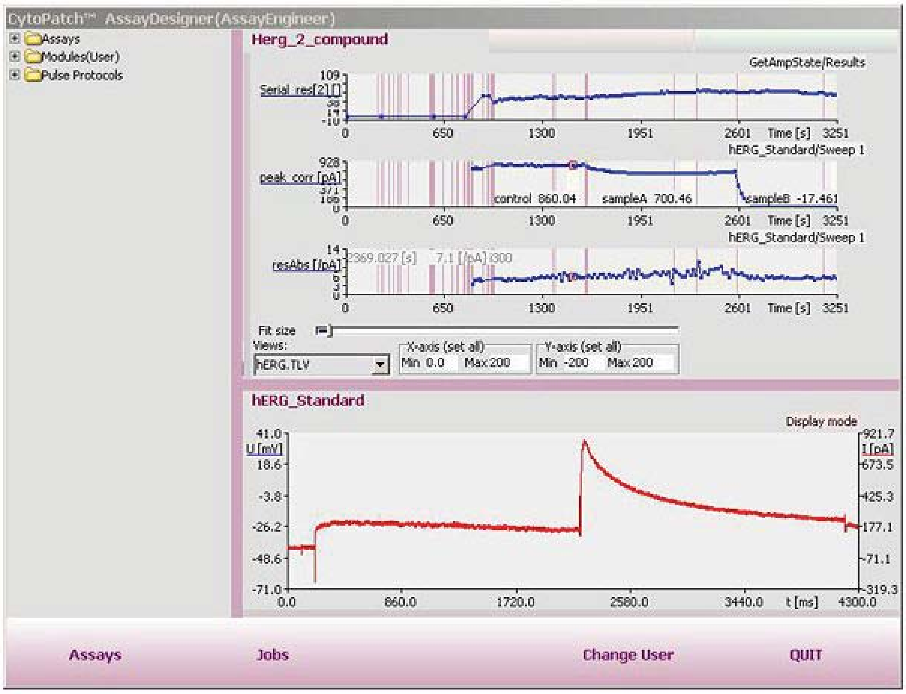
- Timeline functions to monitor experiment progress online
- Temporal analysis and recording quality of parameters of interest

All New Software Interfaces: The Assay Designer

- Intuitive user interface for creating new assays
- Supplied with generic templates for major assay types
- Simple “drag and drop” control of all system components
- Integrated pulse protocol editor

Workflow Manager and CytoPatch™ Software

Two players build one team



The CytoPatch™ Software consists of two main components. First, the central Workflow Manager (WFM) is used to plan, edit and schedule patch clamp studies and furthermore to collect, analyse and archive the data. Second, the CytoPatch™ Software (CPS) runs on each independent CytoPatch™ Instrument and is used to start assays and monitor the recordings.

In the WFM, the compounds and concentrations to be tested and the assay to be used are defined as jobs which are then directed to the individual CytoPatch™ Instrument(s). After receiving the jobs, the CPS controls the respective and entirely automated patch clamp processes running on a single CytoPatch™ Instrument: Chip filling, application of the cells as well as the sealing process, and the establishing of the whole cell configuration are executed automatically. Compensation of electrical capacitances and the serial resistance are accomplished by the CPS controlled patch clamp amplifiers. Test compounds are applied to the cell as defined in the study designed with the WFM software. Raw data and assay results are written to local hard disks and are accessed by the WFM for further analysis and data archiving.

The CPS in turn is installed on each CytoPatch™ Instrument and is used to control the start of the scheduled jobs and also allows monitoring the recordings in real-time.

The Workflow Manager and the CytoPatch™ Software fulfil the requirements of the FDA Regulation 21 CFR 11 with respect to data security, access restriction, audit trail and hardware verification.

Cell Reservoir Improves and Simplifies the Handling of Suspended Cells

Best Suited for Biological Assays requiring Intact Cell Membranes

The Cell Reservoir is a stand-alone bench top storage device that has been designed to maintain the vitality of suspended cells and reduce cell culture work. Due to its compact size, the cell reservoir can be easily placed near the experimental patch clamp setup and be used to store cells in a non-clustered highly vital state and an optimal cell environment for up four hours.

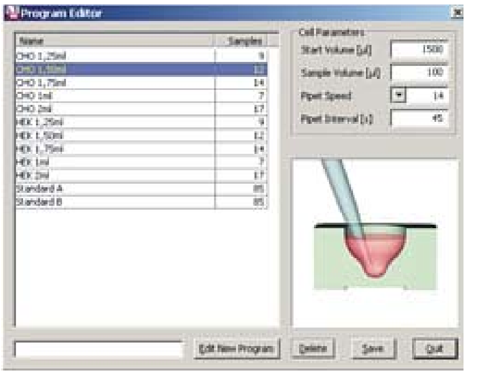
Suspended cells are stored in a Teflon bowl that is mounted into the device. The cell suspension is then aspirated through a low protein binding pipette tip at pre-defined aspirate volumes, pipette speed and pipette intervals. The Cell Reservoir comes with predefined settings that have been optimized for HEK 293 and CHO-K1 cell lines and sample volumes (500 µl to 3700 µl) to maintain the cells at high vitality and low clustering.

Program editing software is also provided and can be used to modify and add maintenance programs. These programs can be simply uploaded into the Cell Reservoir device via USB connection.

The Cell Reservoir facilitates automated and manual patch clamp, high-throughput screening, flow-cytometry and other fluorescence-based assays.

The Cell Reservoir is a storage device for suspended cells. It keeps the cells in a non-clustered state and at high vitality for several hours (> 90 % trypan blue vitality test). It can be placed near the experimental setup.

The cells are supplied in a Teflon bowl that is mounted to the device. Cell suspension is aspirated and dispensed with a pipet tip at pre-defined volumes, pipet speed and pipet intervals.



We recommend the Cell Reservoir to customers who do not use all cells of one preparation at once - e.g., for customers with a manual patch clamp setup.

Ion Channel Cell Lines

Stably Transfected Cell Lines validated for Patch Clamp Assays

The automated patch clamp technology requires high quality cells with respect to membrane stability and ion channel expression rate. Electrophysiologically validated and optimized cells and cell lines are essential for reliable data generation.

We offer the following ion channels as electrophysiologically validated and stably transfected cell lines:

K_V1.x-Channels

(K_V1.1*)
(K_V1.2*)
K_V1.3
K_V1.4
K_V1.5
(K_V1.6*)
hERG - HEK

Further K^+ Channels

K_V10.1 - HEK
(Kir3.1 / Kir3.4**)

Ca-Channels

Ca_V1.2 - HEK
Ca_V2.1 - HEK
Stim1 / ORAI1
(Stim1 / ORAI3**)

K_V7.x-Channels

K_V7.1 / minK
(K_V7.2*)
(K_V7.3*)
(K_V7.5*)
(K_V7.3 / K_V7.2**)
(K_V7.3 / K_V7.5*)

Na_V-Channels

Na_V1.4
Na_V1.5
Na_V1.6
Na_V1.7 - HEK
(Na_V1.8**)

*** : stable cell line not validated**
**** : transient transfection OK**

Trp/P2RXx Cahnnels

TRPV1 - HEK
(TRPV4**)
(TRPV1*)
(P2RX3*)
P2RX7 - HEK

GABA Channels

$GABA_A$ α1 β3 γ2
$GABA_A$ α2 β3 γ2
$GABA_A$ α3 β3 γ2
$GABA_A$ α5 β3 γ2

Further Channels

nAChR alpha 7

Ion Channel	Cell Lines for purchase	Cell Lines under construction
Ca_V1.2		
Ca_V2.1		X
HERG	X	
K_V1.1		X
K_V1.2		X
K_V1.3	X	
K_V1.4		X
K_V1.5	X	
K_V1.6		X
K_V7.3		X
K_V7.5		X
K_V7.3/ K_V7.2		X
K_V7.3/ K_V7.5		X
K_V10.1	X	
K_V7.1/mink		
Kir3.1/Kir3.4		X
nAChR alpha 7	X	
Na_V1.4	X	
Na_V1.5	X	
Na_V1.5	X	
Na_V1.6	X	
Na_V1.7	X	
Na_V1.8		X
P2RX3		X
P2RX7	X	
Stim1/ORAI1		X
Stim1/ORAI3		X
Trp_V1	X	
Trp_V4		X
Trpa1		X

Instant Cells – Frozen Ion Channel Cell Lines for Immediate Use

Functionally Validated for Patch Clamp Electrophysiology

The Instant Cells are ready-to-use within just 15 minutes after thawing. No time-consuming cultivation of the cells is required. Instant Cells allow researchers to start immediately with their assays.

Instant Cells are functionally validated in terms of cell vitality and electrophysiological properties (e.g. seal rate, current amplitude, rundown and whole cell rate) and best suited for manual and automated patch clamp.

Cytocentrics offers Instant Cells expressing relevant ion channels. Now available:

- hERG–HEK 293
- Service: we adapt every cell line to the Instant Cells System

Advantages:

- Reliable data acquisition through quality controlled cells
- Frozen cells are ready-to-use in less than 15 minutes after thawing
- No further cultivation required
- No cell culture lab necessary
- Applicable to automated and manual patch clamp, high- throughput screening, flow-cytometry & other fluorescence- based assays
- Cell Reservoir preserves the cells in a non-clustered state at highest vitality for at least two hours

Cytocentrics' Instant Cells Kit combines stably transfected and ready-to-use cells with a bench-top storage device called Cell Reservoir for the first time. The Instant Cells are a validated system in regards to performance, accuracy and quality. Cytocentrics designed this innovative cell culture kit to simplify the day-to-day cell handling procedure necessary for ion channel screening. It enables researchers to work with frozen cells possessing a constant and equal high quality. The convenient kit with frozen Instant Cells offers highest flexibility: the cells are ready-to-use within just 15 minutes. No time-consuming cultivation of the cells is required - researchers can immediately start with their assays. Highest cell vitality is obtained by transferring the cells into the Cell Reservoir.

The Cell Reservoir is an elegant stand-alone bench-top storage device for suspended cells. It provides an optimal cell environment and preserves the cells in a non-clustered state at a high vitality for at least two hours. This product is the first commercialized Instant Cells Kit in the world. Together with the Cell Reservoir, it helps researchers to improve and simplify cell handling in the drug discovery process.

hERG HEK 293 Instant Cells

The Instant Cells Kit includes:

- Caps with stably transfected hERG-HEK 293 cells (formerly: ERG1, KCNH2, Kv11.1)
- Intracellular solution
- Extracellular solution
- QA certificate
- Cell Reservoir

hERG electrophysiology

Instant HEK 293 cells expressing the human ERG (Ether-a-go-go Related Gene) potassium channel were characterized regarding their electrophysiological and pharmacological properties using conventional whole cell recordings. hERG currents were activated by depolarizing voltage steps beginning at –50 mV. Further depolarization led to a decrease in outward current amplitudes due to the onset of rapid inactivation.

Representative hERG outward currents were recorded from Instant HEK 293 cells stably expressing the hERG ion channel. hERG Currents were elicited upon depolarization of the membrane to +40 mV in 10 mV increments. hERG tail currents were elicited by means of a repolarizing step to -50 mV (2 s).

hERG pharmacology

Dose response data for different compounds were obtained with conventional patch clamp. After whole cell establishment, increasing concentrations of quinidine, cisapride, E4031 and terfenadine were applied to the cells.

Compound	IC50
Quinidine	116.4 nM
Cisapride	12.3 nM
E4031	11.6 nM
Terfenadine	10.8 nM

Contact us

Biotechnology is the technology of the future – Ion Channel Solutions with Cytocentrics GmbH

Cytocentrics provides fully automated patch clamp instruments based on our proprietary technology to obtain physiologically relevant data from cell based assays for ion channel research.

Cytocentrics' GLP compliant products offer simplified workflows, smaller sample sizes, enhanced data quality and reduced costs compared with conventional assay techniques.

Cytocentrics offers both, GLP and non-GLP testing services supporting our clients to discover novel ion channel drugs and expose pharmacological adverse effects faster and more reliably.

Cytocentrics' manual and automated patch clamp screening services are available for a wide range of voltage and ligand gated ion channels, covering all relevant electrophysiology applications.

Our emphasis on high data quality eliminates the risk of identifying a compound as false negative or false positive, and thus minimizes the risk of losing time and money in the drug development process.

Benefit from our broad ion channel screening experience and in vitro target portfolio for safety- and efficacy screening for fast and reliable evaluation of your compounds.

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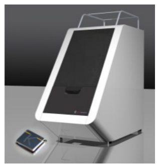
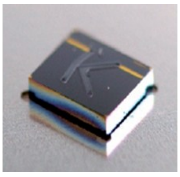
CytoPatch™ technology:

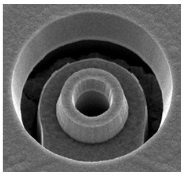
Characterising voltage-gated sodium channels



CytoPatch™ technology produces manual patch clamp quality data from a fully automated system.

The CytoPatch™ is a combination of hardware, software and proprietary microfluidic chip technology that together function to produce manual patch clamp quality data from a fully automated, walk-away, system.





The microfluidic chip incorporating a true giga-seal pipette within a small perfusion "chamber", and the unique continuous perfusion system with fast wash-in/out capability make the CytoPatch™ the perfect patch clamp platform for the study of both ligand- and voltage-gated ion channels in cell lines, stem cells and primary cell preparations.

Functional and pharmacological investigation of voltage-gated sodium channels using CytoPatch™ technology

Voltage-gated sodium channels (Na_v) are encoded by ten genes and are widely distributed in both excitable and non-excitable cells. Essential for action potential generation in the central and peripheral nervous systems, and cardiac and skeletal muscle, Na_v channels are validated therapeutic targets in epilepsy (Na_v1.1), neuropathic pain (Na_v1.7-1.9), cardiac arrhythmias (Na_v1.5) and skeletal myopathies (Na_v1.4).

Here, human embryonic kidney (HEK) 293 cells stably expressing the hNa_v1.5 channel and Chinese Hamster Ovary (CHO) K1 cells stably expressing the hNa_v1.7 channel were used. Cells were cultured under appropriate conditions and harvested according to standard protocols. They were stored in the cell reservoir on the CytoPatch™ Instrument and used for up to 4 h.

Na_v1.7 Na_v1.5 Na_v1.5

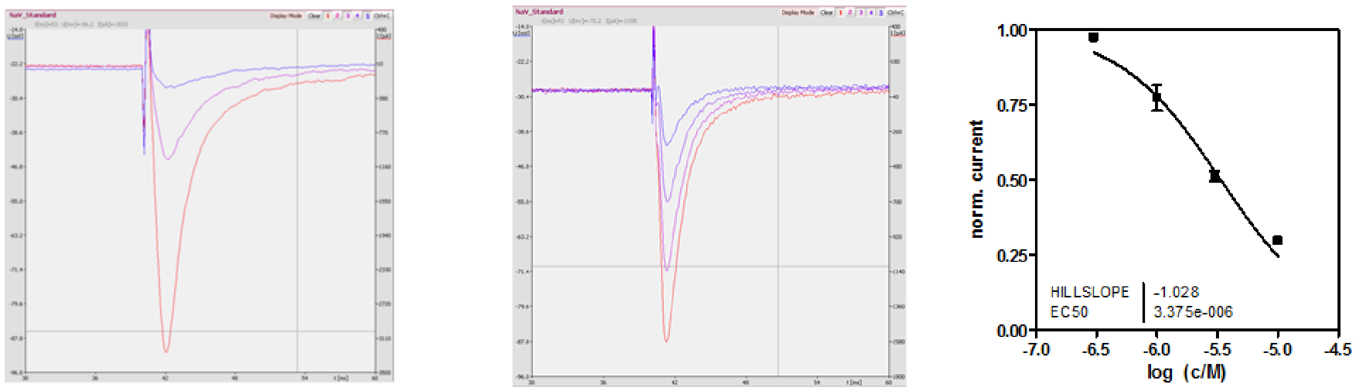


Figure 1. Na_V1.7 and Na_V1.5 can be pharmacologically distinguished. Left: 1 µM TTX almost completely inhibits Na_V1.7, whereas it causes less than 50 % inhibition of Na_V1.5 (middle). Here, almost complete inhibition is observed with a concentration of 10 µM TTX. Right: Concentration-response curve of TTX inhibition of the Na_V1.5 channel. The IC_{50} value was determined to be 3.4 µM.

Na_v1.5 activation

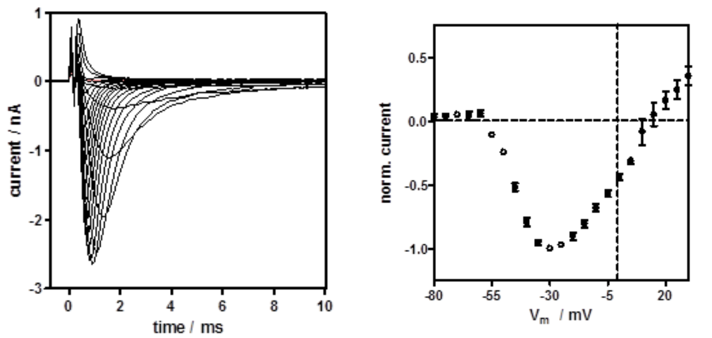


Figure 2. Na_V1.5 whole-cell currents recorded with the CytoPatch™ Instrument. Left: Overlay of current traces showing the responses to 20 ms voltage pulses from □70 mV to +45 mV in 5 mV increments. Right: IV-plot of Na_V1.5 activation (mean ± S.D. of 4 cells; liquid junction potential corrected).

Na_v1.5 steady-state inactivation

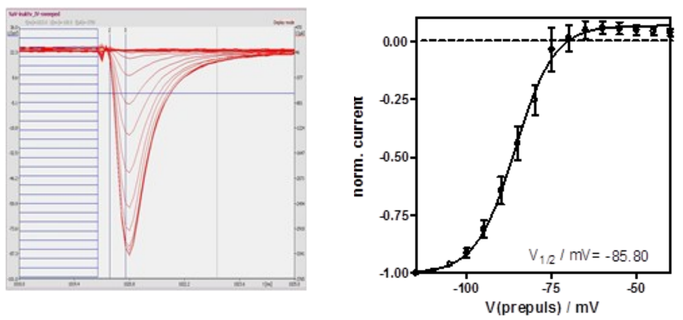


Figure 3. Na_V1.5 steady-state inactivation. Left: Overlay of current traces showing the responses to a 20 ms voltage pulse to 0 mV after conditioning 1 s pre-pulses from -100 mV to 35 mV in 5 mV increments. Right: IV-plot of Na_V1.5 inactivation. Sigmoidal fit of the voltage dependence resulted in a half maximal inactivation voltage ($V_{1/2}$) of -86 mV (mean ± S.D. of 4 cells; liquid junction potential corrected).

Automated voltage-clamp

Whole-cell voltage-clamp recordings were performed on the CytoPatch™ Instrument. After break-through into the attached cell, membrane capacitance and serial resistance were compensated for. To determine TTX effects, cells were clamped from a holding potential (V_{hold}) of -80 mV for 20 ms to -15 mV with a frequency of 0.2 Hz. To investigate use-dependent compound action, a pulse train of 20 pulses to -10 mV (50 ms duration from V_{hold}=-90 mV) with a frequency of 3 Hz was applied before and after compound wash-in.

Data Analysis

Patch clamp data was collected and automatically analysed using the CytoPatchTM Software and the Cytocentrics Work Flow Manager Software. The maximum amplitude of the Na^+ inward current was taken as the output for subsequent analysis. Further statistical analysis was performed using MS Excel and GraphPad Prism 4.

Buffer Composition

The extra cellular buffer (EC) contained in (mM): 28 NaCl, 114 NMDG, 1.2 $CaCl^{2}$*2 H_2O, 1.2 $MgCl_2$*6 H_2O, 0.5 KCl, 18 HEPES; pH adjusted to 7.4 (± 0.1; HCl, NaOH), osmolality adjusted to 315 ± mOsm/l (saccharose). For Na_V1.7, instead of NMDG in total 140 NaCl was used.

The intracellular buffer (IC) contained in (mM): 100 K-Gluconat, 20 KCl, 1 $CaCl_2$, 1 $MgCl_2$, 10 HEPES, 11 EGTA, 4 ATP-Mg^{2+}, 3 Phosphocreatine-Na_2-H_2O, 9 Sucrose. pH 7.2 +/- 0.1; osmolality 295 +/- 5 mOsmol/l.

Compound Handling

Blow fish toxin tetrodotoxin (TTX; Tocris), quinidine and mexiletine (Sigma -Aldrich) were dissolved in water. Aliquots of the stock solutions were stored at -20 °C. The stock solutions of the compounds were thawed once to prepare work solutions in the appropriate EC.

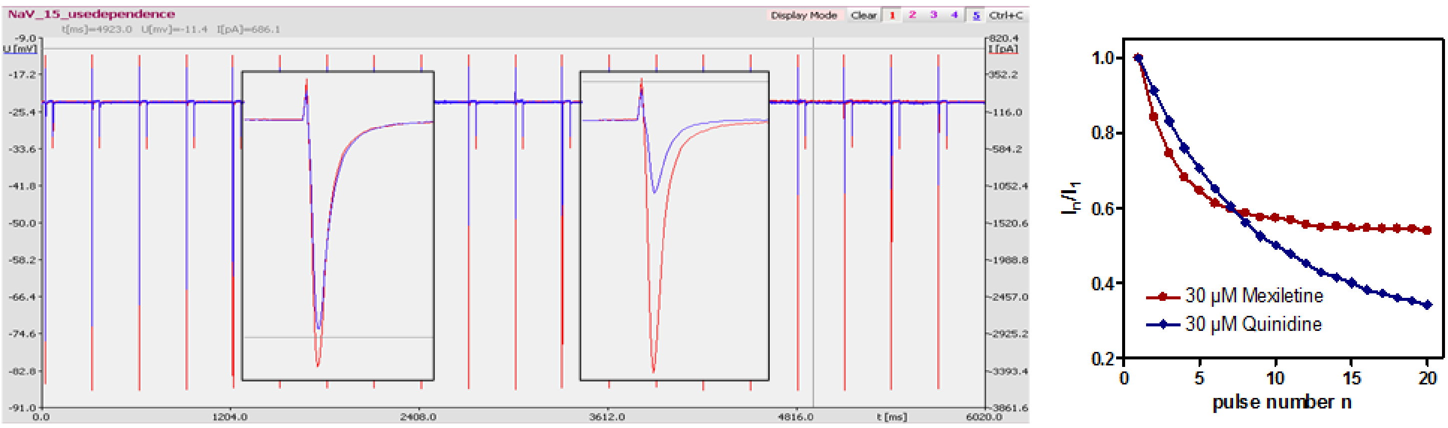


Figure 4. Use-dependent inhibition of Na_V1.5 by 30 µM quinidine: Left: From a holding potential of -90 mV cells were activated by a train of twenty 50 ms voltage-pulses to -10 mV with a 3 Hz repetition frequency. Na_V1.5 currents in response to this voltage protocol before (red) and after compound wash-in (blue). In the inserts the first and last pulse of the sweep trains are enlarged. Analysis of current traces is fully automated in the CytoPatch™ and Work Flow Manager Software. For further statistical analysis, means and S.D. of the concentration replicates can easily be exported to the clipboard. Right: The antiarrhythmics quinidine and mexiletine show different kinetics of use-dependent development of Na_V1.5 inhibition.

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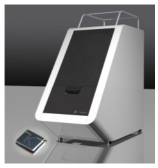
The CytoPatch™ technology and its use in investigating voltage-gated *Shaker*-related potassium channels



The CytoPatch™ technology has been developed to produce manual patch clamp quality data from a fully automated system.

The CytoPatch™ is a combination of hardware, software and proprietary microfluidic chip technology that together function to produce manual patch clamp quality data from a fully automated, walk-away, system.
The microfluidic chip incorporating a true giga-seal pipette within a small perfusion "chamber", and the unique continuous perfusion system with fast wash-in/out capability make the CytoPatch™ the perfect patch clamp platform for the study of both ligand- and voltage-gated ion channels in cell lines, stem cells and primary cell preparations.

Functional and pharmacological investigation of *Shaker*-related potassium channels with CytoPatch™ technology

Members of the shaker-type K channels (K_v1.1 to K_v1.8) play important roles in several physiological processes , e.g., K_v1.3 is highly expressed in human lymphocytes. K_v1.3 is widely regarded as a promising new target for immunosuppression. Inhibitors of K_v1.3 may be useful immunomodulators for the therapy of autoimmune disorders such as multiple sclerosis or Type I diabetes. The CytoPatch™ is capable of investigating biophysical properties and pharmacology of K_v1 channels with the same quality and information content as manual patch clamp.

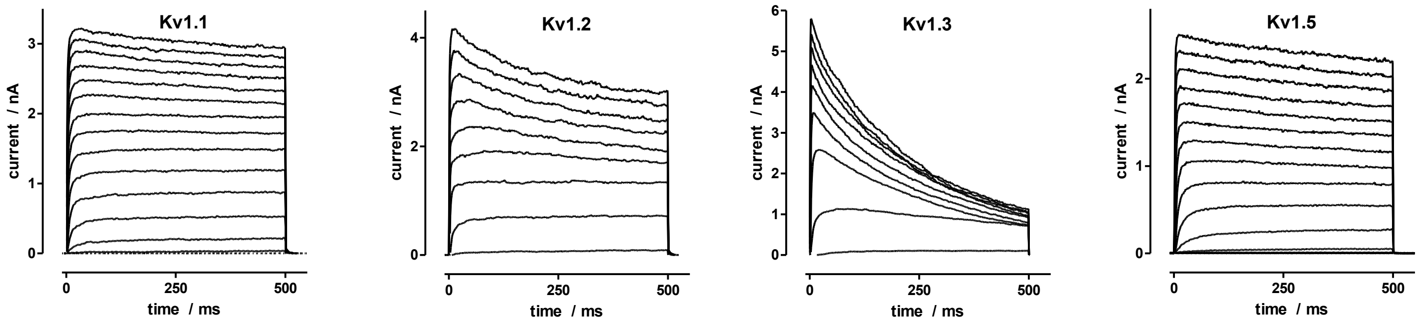


Figure 1. Whole-cell currents recorded from CHO-K1 cells stably expressing the Kv1.1, Kv1.2, Kv1.3 or Kv1.5 ion channel. Overlay of current traces activated by 500ms voltage pulses incremented in 10 mV or 15mV steps from –70mV to +80mV.

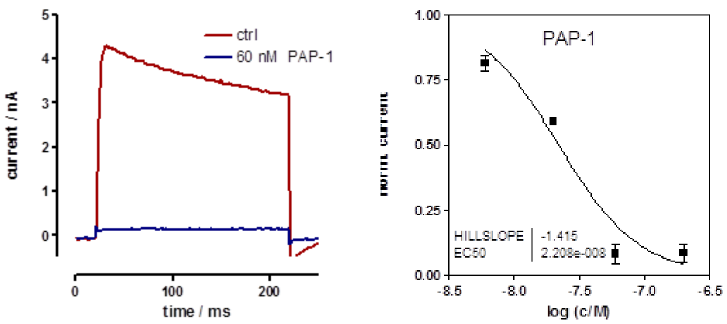


Figure 2. K_v1.3 inhibition by PAP-1. Representative whole-cell K_v1.3 current traces under control conditions (red) and after 8 minute wash-in of 60nM PAP-1 (blue) recorded with the CytoPatch™ (left). Concentration response relationship for the inhibition of K_v1.3 currents by PAP-1. KF based intracellular buffer was used. IC_{50} was determined to be 22nM (right).

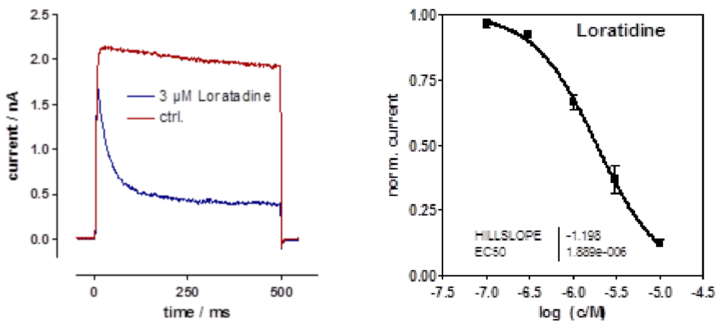


Figure 3. K_v1.5 inhibition by Loratadine. Representative whole-cell K_v1.5 current traces under control conditions (red) and after wash-in of 3µM Loratadine (blue) recorded with the CytoPatch™ (left). Concentration response relationship for the inhibition of K_v1.5 currents by Loratadine. K-Glu based intracellular buffer was used. IC_{50} was determined to be 1.8µM (right).

Automated voltage-clamp

Whole-cell voltage-clamp recordings were performed on the CytoPatch™. After breakthrough into the attached cell, membrane capacitance and serial resistance were compensated for.

Data Analysis

Patch clamp data was collected and automatically analysed using the CytoPatch™ Software and the Cytocentrics Work Flow Manager Software. Further statistical analysis was performed using MS Excel and GraphPad Prism 4.

Buffer Composition

The extra cellular buffer (EC) contained in (mM):
140 NaCl, 2.5 KCl, 2 $MgCl_2$, 2 $CaCl_2$, 10 HEPES, 10 glucose, and 15 sucrose. pH was adjusted to 7.4, the osmolality was 320 mOsm/kg. The buffer was stored at 4 °C, degassed and heated up to room temperature prior to use.

Two different intracellular buffers (IC) were used, consisting of (in mM):
K-Gluconate IC: K-Gluconate 100; KCl 20; $CaCl_2$* 2 H_2O 1; $MgCl_2$* 6 H_2O 1; EGTA 11; Hepes 10; GSH 1; ATP-Mg_2 2; pH 7.2.
K-Fluoride IC: KF 155; $MgCl_2$ 2; HEPES 10, EGTA 10; pH 7.2

Both ICs gave good success rates (K_V1.3-CHO-K1):

K-Glu: 100 % Seal, median R_{seal}: 2.5 GOhm, 92 % Whole-cell, median R_m: 2.7 GOhm (n=161 cells)
K-Fluoride: 91 % Seal, median R_{seal}: 3.2 GOhm, 87 % Whole-cell, median R_m: 1 GOhm (n=599 cells)

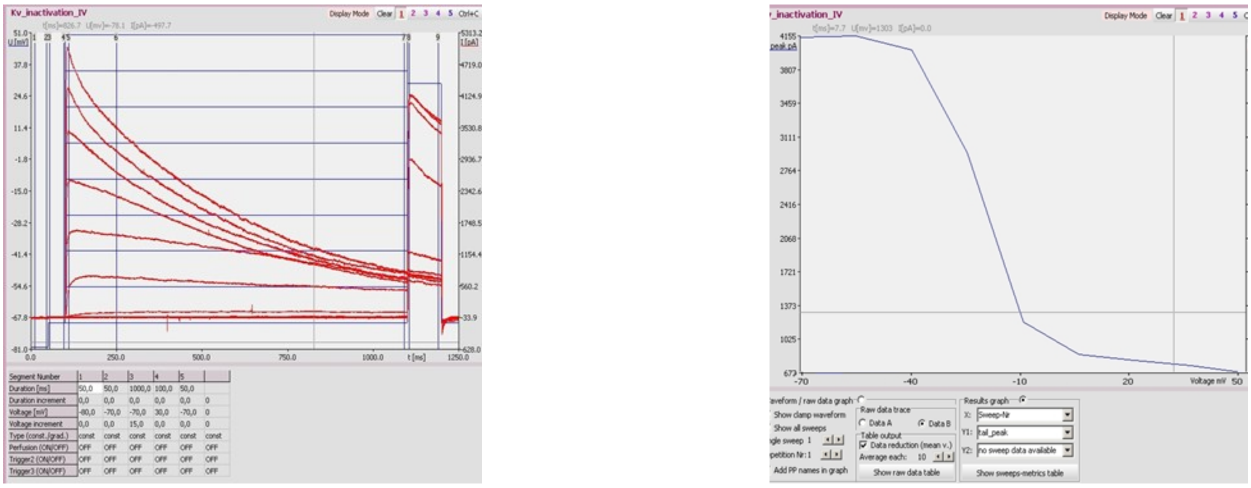


Figure 4. The CytoPatch™ software offers a flexible and powerful pulse protocol editor and online analysis capability. Shown here is the activation pattern to investigate the steady-state inactivation of the K_v1.3 channel (whole-cell recordings, KF IC buffer).

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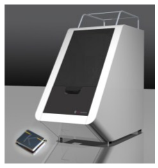
CytoPatch^{TM} technology:

Recording calcium release activated Ca^{2+} channels (CRAC)



CytoPatch^{TM} technology produces manual patch clamp quality data from a fully automated system.

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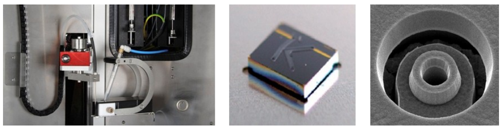

The microfluidic chip incorporating a true giga-seal pipette within a small perfusion "chamber", and the unique continuous perfusion system with fast wash-in (<5ms)/out (<10ms) capability make the CytoPatch^{TM} the perfect patch clamp platform for the study of both ligand- and voltage-gated ion channels in cell lines, stem cells and primary cell preparations.

Calcium release activated Ca^{2+} currents I_{CRAC}

Calcium (Ca^{2+}) release-activated Ca^{2+} channels (CRAC) are highly Ca^{2+}-selective, low-conductance channels known to mediate extracellular Ca^{2+} influx in response to depletion of intracellular Ca^{2+} stores. As the first identified and best studied of store-operated Ca^{2+} channels, the molecular mechanism linking Ca^{2+} influx via activated CRAC channels to changes in endoplasmic reticulum (ER) intracellular Ca^{2+} stores has only recently been discovered. Involved is the coupling of two transmembrane proteins: 1) an ER intracellular Ca^{2+} store sensor (stromal interaction proteins STIM1 or STIM2); and 2) a plasma membrane ion channel pore-forming subunit (Orai1, Orai2 or Orai3), with STIM1 and Orai1 identified as underlying CRAC channel function. Essential for Ca^{2+} homeostasis, CRAC pathologic dysfunction contributes to such diseases as immune deficiency syndrome, atheroscleosis, bronchoconstriction, erectile dysfunction and breast cancer.

The CytoPatch™ can be used to study store depletion induced either by passive depletion using an IC containing Ca^{2+} chelators like EGTA or BAPTA or by active depletion using the IP3-receptor agonist InsP3, as well as inhibition of Ca^{2+} re-uptake into intracellular Ca^{2+} stores by application of thapsigargin, a blocker of the sarcoplasmic/ER Ca ATPase (SERCA).

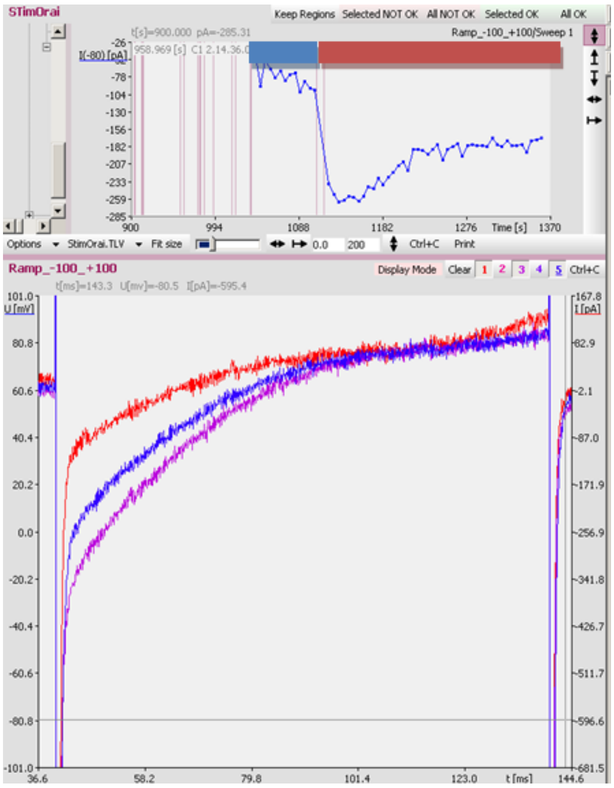

Figure 1. I_{CRAC} whole-cell currents recorded with the CytoPatch^{TM} Instrument. The upper curve shows the time line view of the whole-cell current response to the voltage ramp measured at -80 mV. Blue bar indicates cell perfusion with EC containing 2 mM Ca^{2+}, red bar indicates perfusion with EC containing 10 mM Ca^{2+} and 40 µM thapsigargin (TG). Original traces are depicted showing the whole-cell current at the beginning of the experiment (red), at maximal activation in the presence of EC with 10 mM Ca^{2+} and TG (purple) and after 5 min wash-in of this EC (blue).

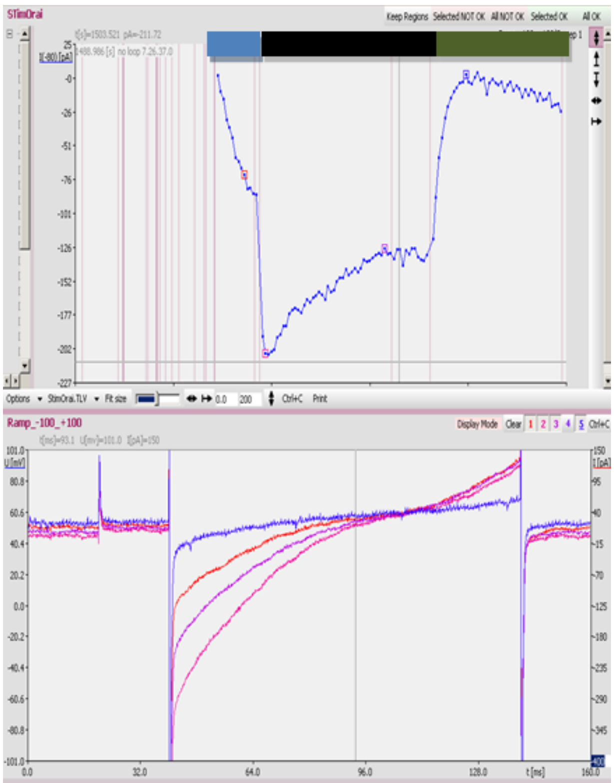

Figure 2. Inhibition of I_{CRAC} by SKF-96365 recorded with the CytoPatch^{TM} Instrument. The upper curve shows the time line view of the whole-cell current response to the voltage ramp measured at -80 mV. Blue bar indicates cell perfusion with EC with 2 mM Ca^{2+}, black bar indicates perfusion with EC containing 10 mM Ca^{2+}. Green bar indicates the wash-in of EC with 10 mM Ca^{2+} containing additionally 50 µM SKF-96365. Original traces are depicted showing the whole-cell current at the beginning of the experiment (red), at maximal activation in the presence of EC with 10 mM Ca^{2+} (pink), after 5 min wash-in of this EC (purple) and after wash-in of SKF-96365 (blue). The IC contained 15 mM BAPTA and 30 µM $InsP_3$.

Cells

Here, Chinese hamster ovary (CHO) K1 cells stably over expressing STIM1/Orai1 were used. Cells were cultured under appropriate conditions and harvested according to standard protocols. The cells were stored in the cell reservoir of the CytoPatch™ Instrument and used for up to 4 h.

Automated voltage-clamp

Whole-cell voltage-clamp recordings were performed on the CytoPatch™. After successful establishing of the whole-cell configuration, a ramp protocol from -100 mV to 100 mV in 100 ms was applied every 5 s. During the sealing process and up to one minute after whole-cell breathrough, cells were continuously perfused with an extra cellular buffer (EC) containing 2 mM Ca^{2+}. Then, the cells were perfused with an EC containing 10 mM Ca^{2+} for 5 min. After this control phase, the same EC containing SKF-96365 was applied for another 5 min.

Data Analysis

Patch clamp data was collected and automatically analysed using the CytoPatch™ Software and the Cytocentrics Work Flow Manager Software. The current amplitude of the STIM/Orai1 mediated inward current at a membrane potential of –80mV was taken as readout. Further statistical analysis was performed using MS Excel and GraphPad Prism 4.

Buffers and Compounds

Buffers: The extra cellular buffer (EC) for preparation and storage of the cells and for the initial phase of the patch clamp recordings contained in (mM): 140 NaCl, 2.5 KCl, 2 $MgCl_2$, 2 $CaCl_2$, 10 HEPES, 10 Glucose, 15 Sucrose; pH: 7.4 +/- 0.1; osmolality 320 +/- 5 mOsmol/l. The high Ca^{2+} EC contained (in mM): 4 KCl, 120 NaCl, 10 $CaCl_2$, 1.2 $MgCl_2$, 10 HEPES, 20 D(+)-glucose, 10 saccharose. pH 7.4; osmolality 320 +/- 5 mOsmol/l.

The intracellular buffer (IC) contained in (mM): 145 Glutamate, 8 NaCl, 1 $MgCl_2$, 10 HEPES, 10 EGTA. BAPTA and $InsP_3$ were added as indicated. pH 7.2 adjusted with CsOH. pH: 7.2 +/- 0.1; osmolality 295 +/- 5 mOsmol/l.

The SERCA inhibitor Thapsigargin (TG; Ascent Scientific) was dissolved in DMSO. The unselective Ca^{2+} channel inhibitor SKF-96365 (Ascent Scientific) was dissolved in aqua dest. Aliquots of both compounds were stored at -20 °C. Prior the experiments stocks were thawed and diluted in the appropriate EC.

Summary

Here we show that the automated patch clamp platform CytoPatch™ Instrument is well suited to investigate Ca^{2+} release activated Ca^{2+} currents. Active depletion of intra-cellular Ca^{2+} stores using an IC containing 30 µM $InsP_3$ in combination with BAPTA present in a concentration of 10 to 15 mM led to robust and reproducible activation of I_{CRAC}. By application of the unselective Ca^{2+} current inhibitor SKF-96365 it was proven that pharmacological effects on I_{CRAC} can be investigated with this system.

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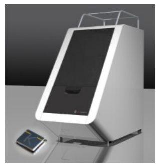
CytoPatch™ technology:

hERG ion channel safety pharmacology



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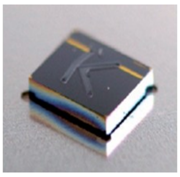
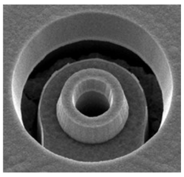




The microfluidic chip incorporates a real patch pipette within a small perfusion “chamber”, and the unique continuous perfusion system with fast wash-in/out capability make the CytoPatch™ the perfect patch clamp platform for the study of both ligand- and voltage-gated ion channels in cell lines, stem cells and primary cell preparations.

hERG screening in safety pharmacology using CytoPatch™ technology

The slowly activating and deactivating voltage-gated K^+ channel, $K_v11.1$ (KCNH2), commonly known as hERG (*human* ether-à-go-go-Related gene), underlies the cardiac rapid delayed rectifier current I_{Kr}. Functioning hERG channels are essential for the early phase of normal cardiac action potential repolarization. Although additionally expressed in brain, smooth muscle, endocrine cells as well as numerous tumor-derived cell lines, hERG is best characterized in heart where its lack of function, as in the inherited long QT syndrome (LQTS), produces life threatening ventricular arrhythmias and sudden cardiac death. Drugs that block hERG can prolong the QT interval inducing LQTS-like symptoms that increase the risk of cardiac arrest. As a consequence, FDA guidelines require drugs to be tested against hERG.

Using Cytocentrics’ HEK-293 hERG Instant Cells for optimal assay standardization and repeatability we show concentration response relationships of typical hERG blocking compounds and compare with data generated with the manual patch clamp technique.

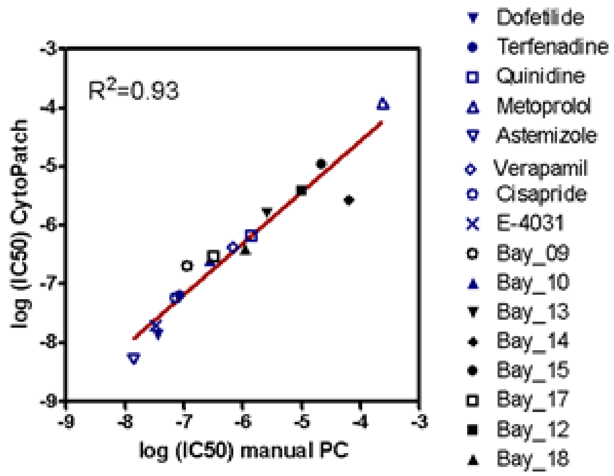
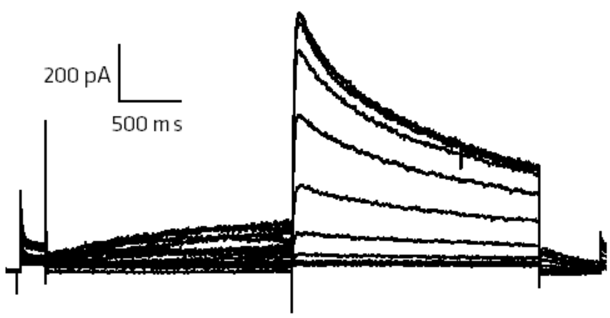


Figure 1. Blinded hERG Study with compounds provided by Bayer Schering Pharma (BSP)

18 blinded compounds were assessed for their impact on the cardiac human Ether-à-go-go related gene K+ channel. The IC_{50} values obtained by the CytoPatch™ Instrument using the frozen human embryonic kidney 293 cells showed a high correlation (R2 = 0.928) with those obtained from manual patch clamp recordings with human embryonic kidney 293 cells from a running cell culture. Moreover, this correlation extended to sticky compounds such as terfenadine or astemizole.

Scheel O, Himmel H, Rascher-Eggstein G, Knott T. (2011): Introduction of a modular automated voltage-clamp platform and its correlation with manual human Ether-à-go-go related gene voltage-clamp data. Assay Drug. Dev. Technol. 9(6):600-607



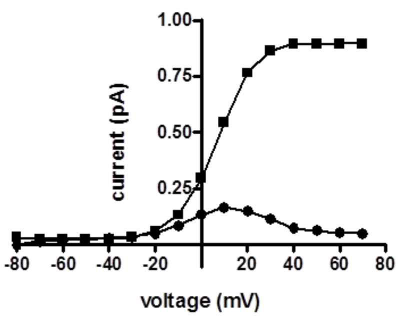


Figure 2. Left: Representative hERG outward currents recorded from HEK 293 Instant Cells stably expressing the hERG ion channel. Right: Example for isochronal I/V relationship of the hERG activation current (dots) and I/V relationship of hERG tail current (squares) (not corrected for LJP).

Automated voltage-clamp

Whole-cell voltage-clamp recordings were performed on the CytoPatch™. After breakthrough into the attached cell, membrane capacitance and serial resistance were compensated for. To determine compound effects, cells were clamped from a holding potential (V_{hold}) of -70 mV, executing the following pulse protocol every 10 s: for 100 ms to –50 mV followed by a 2 s step to +40 mV. To evoke outward tail currents, a 2 s step to -50 mV followed. The peak tail current was corrected for the leak current determined during the first short voltage step to –50 mV.

Data Analysis

Patch clamp data was collected and automatically analysed using the CytoPatch™ Software and the Cytocentrics Work Flow Manager Software. The maximum hERG tail current amplitude current was taken as the output for subsequent analysis. Further statistical analysis was performed using MS Excel and GraphPad Prism 4.

Buffer Composition

Buffers: The extra cellular buffer (EC) contained in (mM): 140 NaCl, 2.5 KCl, 2 $MgCl_2$, 2 $CaCl_2$, 10 HEPES, 10 glucose, and 15 sucrose. pH was adjusted to 7.4, the osmolality was 320 mOsm/kg. The buffer was stored at 4 °C, degassed and heated up to room temperature prior to use.

The intracellular buffer (IC) contained in (mM): 100 KGluconat, 20 KCl, 1 $CaCl_2$, 1 $MgCl_2$, 10 HEPES, 11 EGTA-KOH, 4 MgATP, 3 phosphocreatine -Na_2-H_2O, and 9 sucrose. pH was adjusted to 7.2, the osmolality was 295 mOsm/kg. Aliquots were stored at -20 °C, thawed prior to use, and used for maximum 4 h.

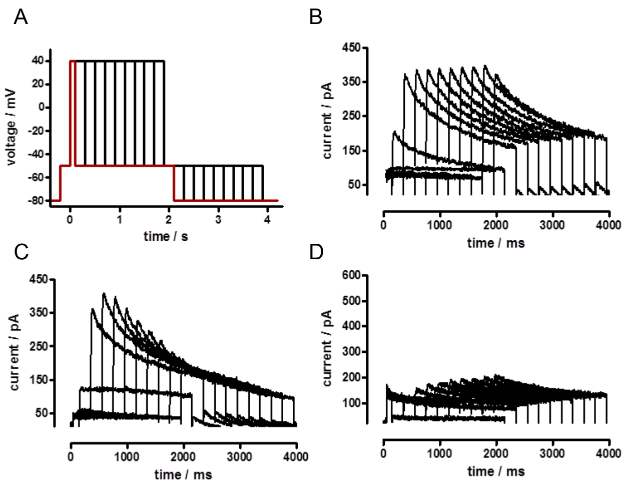


Figure 3. Envelope of tails protocol to determine blocking mechanism of compounds:
A: hERG tail currents are elucidated by activating depolarizing pulses with increasing durations. For open channel blockers channel inhibition increases with the number of pulses and thus with the time the channel is pushed in an open state while closed channel blockers would not show a strong channel open time dependence.
B: hERG tail currents evoked by an envelope of tails protocol. Under control conditions tail current amplitude reaches saturation after the third depolarizing voltage pulse.
C: Astemizole is a typical open channel hERG blocker: Time dependent onset of channel inhibition by astemizole is seen in the hERG tail currents evoked by the envelope of tails protocol.
D: Fluvoxamine inhibits hERG in the closed channel state: No time dependence of channel inhibition by fluvoxamine is seen in the hERG tail currents evoked by the envelope of tails protocol.

Compound Handling

All compounds were obtained from commercial suppliers or are proprietary structures synthesized at BSP. Water-insoluble compounds were dissolved in dimethylsulfoxide (DMSO) and aliquots were stored at -20 °C. The stock solutions were further diluted in DMSO and then diluted 1:1000 in EC buffer, yielding a final compound concentration of 0.1 % DMSO. Water-soluble compounds as quinidine were dissolved in water. Aliquots of the stock solutions were stored at -20 °C. The stock solutions of the compounds were thawed once to prepare work solutions in the appropriate EC.

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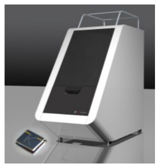
Agonist and antagonist action on the $GABA_A$ receptor measured using Cytopatch™ technology.



CytoPatch™ technology produces manual patch clamp quality data from a fully automated system.

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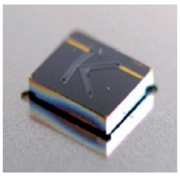

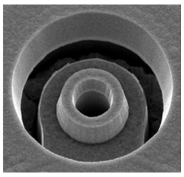

The microfluidic chip incorporates a real patch pipette within a small perfusion "chamber", and the unique continuous perfusion system with fast wash-in/out capability make the CytoPatch™ the perfect patch clamp platform for the study of both ligand- and voltage-gated ion channels in cell lines, stem cells and primary cell preparations.

Validation of the agonist and antagonist assays on the Cytopatch™ using the γ-aminobutyric acid type A ($GABA_A$) ion channel.

$GABA_A$ receptors are ligand-gated ion channels that mediate γ-aminobutyric acid (GABA)-induced inward chloride (Cl^-) currents arising from Cl^- efflux. Ubiquitously expressed throughout the central nervous system (CNS), $GABA_A$ receptors are expressed in 20-30% of CNS neurons and are located on neuronal somatic, dendritic and axon initial segment membranes. $GABA_A$ receptors induce hyperpolarization of the transmembrane potential and underlie CNS fast and slow inhibitory postsynaptic potentials resulting from GABA-mediated neurotransmission. As heteropentamers, $GABA_A$ receptors are assembled from a family of 19 different subunits (α1-6, β1-3, γ1-3, δ, ε, θ, π, and ρ1-3) with the major isoforms containing α, β and γ subunits. Therapeutic applications for $GABA_A$ receptor modulation include classic benzodiazepine applications for anxiety disorders and insomnia, as well as potential clinical use in analgesia, cognitive deficits, schizophrenia, depression and stroke.

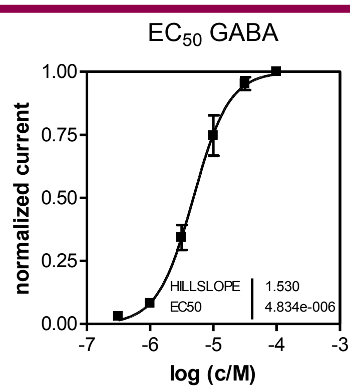

Figure 1. The concentration-response curve for GABA as fitted using a sigmoidal dose-response algorithm (variable slope). The mean EC_{50} value for GABA was 4.8 µM (n=5), the Hill coefficient 1.53.

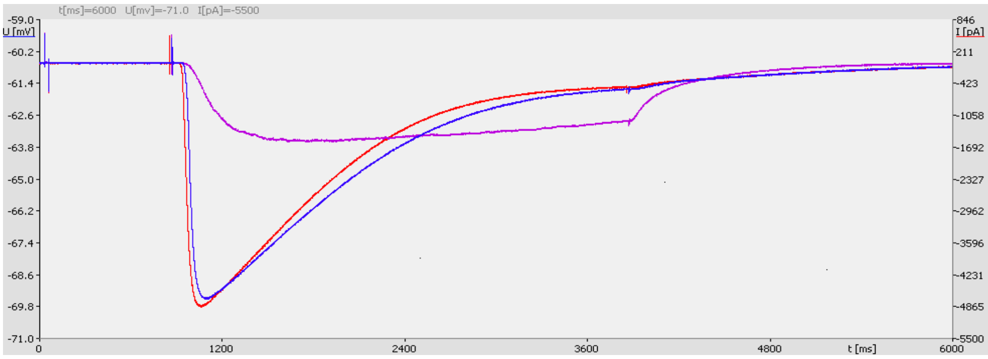

Figure 2. Concentration-dependent GABA-response: Wash-in of 100µM GABA (red), followed by a wash-in of 1µM GABA (purple), followed by a wash-in of 100µM GABA (blue).

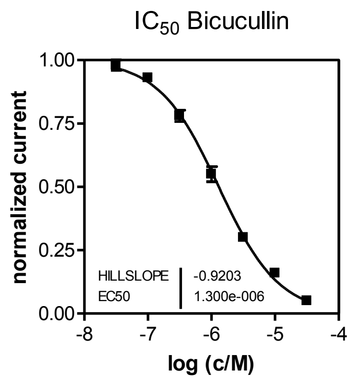

Figure 3. Concentration-response curves for Bicuculline were fitted using a sigmoidal dose-response algorithm (variable slope). The mean EC_{50} value for Bicuculline was 1.3 µM (n=4), the Hill coefficient -0.92.

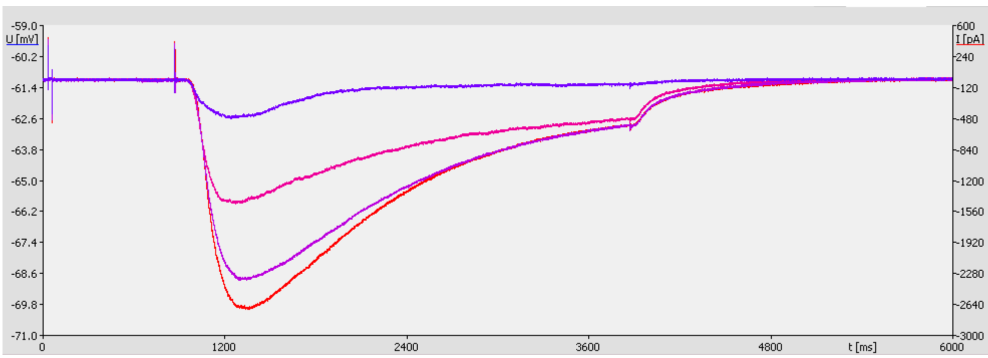

Figure 4. Action of the $GABA_A$ receptor antagonist Bicuculline: Wash-in of 5µM GABA (pink), followed by a wash-in of 5µM GABA/0,1µM Bicuculline (pink), followed by a wash-in of 5µM GABA/1µM Bicuculline (red), followed by a wash-in of 5µM GABA/10µM Bicuculline (purple).

Cell Culture Conditions

Here, Chinese Hamster Ovary (CHO) K1 cells stably expressing the $GABA_A$ were used. Cells were cultured under appropriate conditions and harvested according to standard protocols. After resuspension in extracellular buffer ($2x10^6$ cells/ml) they were stored in the cell reservoir on the CytoPatch™ Instrument and used for up to 4 h.

Automated Voltage-Clamp

Whole-cell voltage-clamp recordings were performed on the CytoPatch™ Instrument. After break-through into the attached cell, membrane capacitance and serial resistance were compensated for and cells clamped at -80 mV. Cells were exposed to GABA or GABA/Bicuculline for 3 s. Up to seven compound concentrations were applied to a single cell. To determine the GABA EC50, a maximum GABA concentration off 100µM was used as a control in the first, third and seventh application. To determine the Bicuculline IC50, a concentration of 5µM GABA was used as a control in the first, third and seventh application.

Compound Handling

γ-aminobutyric acid (GABA) (Sigma-Aldrich) was dissolved in water at a concentration of 1 M. Aliquots of the stock solutions were stored at -20°C. The stock solution was thawed once to prepare the following work solution concentrations: 100 µM, 31.6 µM, 10 µM, 3.16 µM, 1 µM, 0.316 µM.

Bicuculline (FLUKA) was dissolved in DMSO at a concentration of 100 mM. Aliquots of the stock solutions were stored at -20 °C and defrosted once to prepare the working solutions. After thawing, the stocks were further diluted in DMSO. As a final step the intermediate stocks were diluted 1:1000 in extracellular buffer containing 5 µM GABA, yielding the correct Bicuculline concentration of 10 µM, 3.16 µM, 1 µM, 0.32 µM, 0.1 µM, 0.03 µM in 0.1% DMSO.

Data Analysis

Patch clamp data was collected and automatically analysed using the Cytopatch™ Software and the Cytocentrics Work Flow Manager Software. The maximum amplitude of the GABA current was taken as the output for subsequent analysis. Further statistical analysis was performed using MS Excel and GraphPad Prism 4.

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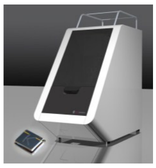
CytoPatch™ technology:

Cardiac safety pharmacology



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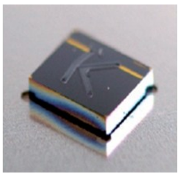
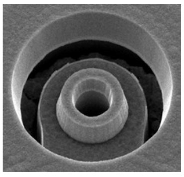

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Ion channel screening in safety pharmacology using CytoPatch™ technology

Many potential drugs fail during the drug development process due to safety and toxicity issues. With cardiotoxicity ranking as the most prominent reason, FDA guidelines require drugs to be tested against the cardiac hERG (*human* ether-à-go-go-Related gene) channel. Known drug-induced fatal arrhythmias include prolongation of the cardiac QT interval and torsades de pointes (polymorphic ventricular tachycardia). Drugs that block normal repolarization of the cardiac action potential can be proarrhythmic by inhibiting the underlying rapid (I_{Kr}) and slow (I_{Ks}) delayed rectifier potassium currents. hERG ($K_v11.1$ or KCNH2) channels underlie I_{Kr} while I_{Ks} consists of $K_v7.1$ (KCNQ1) channel subunits co-assembled with MinK (KCNE1) b-subunits. Other dominant channels in human cardiomyocytes that contribute to cardiac arrhythmias include the rapidly inactivating $Na_v1.5$ (SCN5A) sodium channel and the slowly inactivating L-type calcium channel, $Ca_v1.2$ (CACNA1C). The pro- versus anti-arrhythmic potential of drug interactions with hERG, $K_v7.1$/MinK, $Na_v1.5$ and $Ca_v1.2$ cardiac channels are complicated by numerous channelopathies associated with various cardiac diseases and disorders.

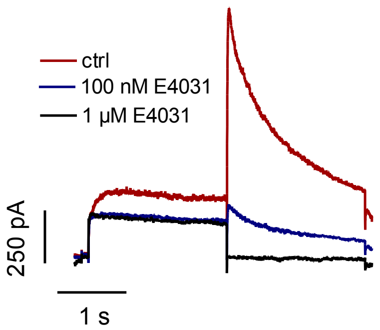


Figure 1: Fig. 1: Whole-cell current traces of HEK293 cells expressing the hERG ion channel recorded with the CytoPatch™ under control conditions (red trace) and after wash-in of 100 nM (blue trace) and 1 µM (black trace) E-4031.

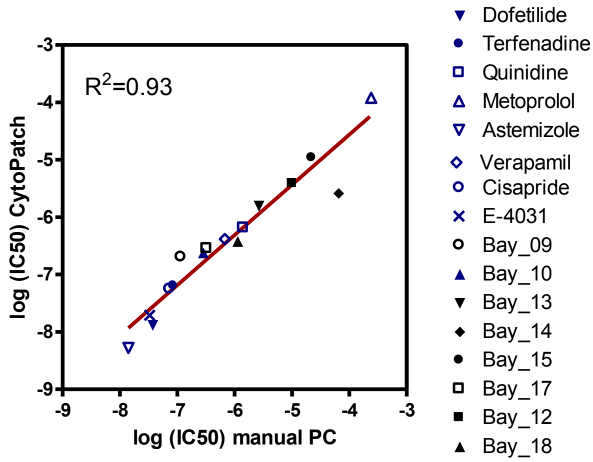


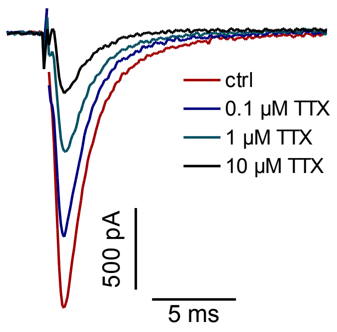
Figure 2: Inhibition of hERG whole-cell currents by blinded compounds provided by Bayer Schering Pharma (BSP) determined with the CytoPatch™ in correlation with manual patch clamp data obtained at BSP. Adopted from: Scheel et al.: Assay Drug. Dev. Technol. 2011, 9(6):600-607

Automated voltage-clamp

Whole-cell voltage-clamp recordings were performed on the CytoPatch™. After breakthrough into the attached cell, membrane capacitance and serial resistance were compensated for. Ionic currents were activated with the appropriate voltage stimulation patterns. Standard electrophysiological buffers were used as described in the relevant literature. For $Na_v1.5$ recordings Na^+ was substituted by NMDG to scale down current amplitudes.

Data Analysis

Patch clamp data was collected and automatically analysed using the CytoPatch™ Software and the Cytocentrics Work Flow Manager Software.



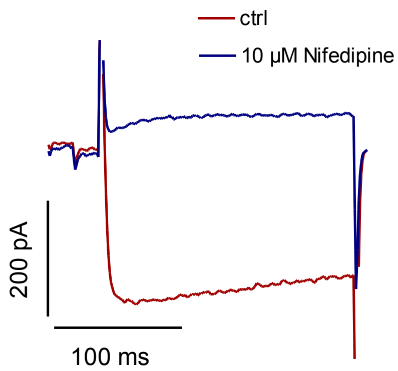
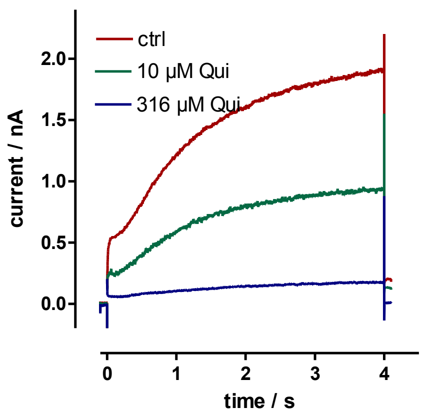


Fig. 3: Whole-cell current traces of HEK293 cells expressing the $Na_V1.5$ ion channel recorded with the CytoPatch™ under control conditions (red trace) and after wash-in of increasing concentrations of tetrodotoxin (TTX).

Fig. 4: Whole-cell current traces of HEK293 cells expression the cardiac L-type Ca^{2+} channel complex consisting of the subunits $Ca_V1.2$/b2/a2d1**.** Currents were recorded with the CytoPatch™ under control conditions (red trace) and after wash-in of 10 µM nifedipine (blue trace).



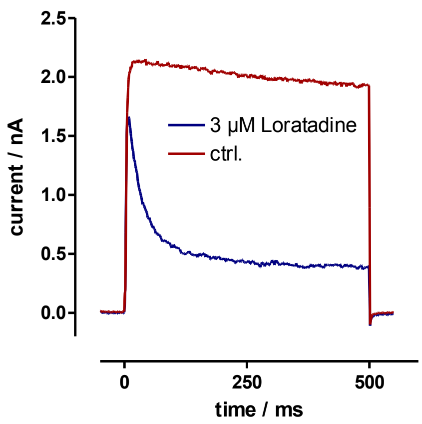


Fig. 5: Whole-cell current traces of HEK293 cells expressing the $K_v7.1$/ minK ion channel recorded with the CytoPatch™ under control conditions (red trace) and after wash-in of increasing concentrations of quinidine.

Fig. 6: Whole-cell current traces of CHO-K1 cells expressing the $K_v1.5$ channel recorded with the CytoPatch™ under control conditions (red trace) and after wash-in of 3 µM Loratadine.

Summary

These studies show that the CytoPatch™ is ideally suited for low-mid throughput laboratories investigating drug effects on cardiac ion channels in a fully automated fashion as it delivers data of equal quality to the "gold standard" of manual patch clamp and has the assay flexibility to record from a broad range of ion channels and multiple cell types.

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