

Cell- and Tissue-Based Electrophysiological Assays for Drug Testing

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Electrophysiology which treats of electrical phenomena produced by or in living organisms, has obtained increased importance in the field of drug discovery due to two facts:

It is believed that the human genome encodes more than 300 different ion channels and an increasing number of diseases has been shown to be related to dysfunction of this membrane proteins or affected regulatory pathways (Lehmann-Horn & Jurkat-Rott, 1999, Willumsen et al., 2003). Consequently, drug discovery nowadays is closely related with the screening of large numbers of compounds against multiple ion channel targets. The other driving force is safety pharmacology, which is defined as “those studies that investigate the potential undesirable pharmacodynamic effects of a substance on physiological functions” (ICH S7A Document, www.ich.org). As cardiac and non-cardiac drugs in the past have affected cardiac function, safety pharmacology is supposed to include electrophysiological studies for assessing the potential of pharmaceuticals for delayed ventricular repolarization (QT interval prolongation) by human pharmaceuticals (ICH S7B Document)

The resulting need for appropriate test assays has forced the ongoing development of electrophysiological methods, instrumentation and cell- and tissue based assays that enable parallel and automated monitoring of ion channel activity as a measure for the pharmacological activity of a compound (Stett et al., 2003b, Worley, 2003).

Automated Patch-Clamping. The preferred method with the highest information content for characterisation of ion channel function and its regulation is the patch-clamp technique that allows reliable recording of ionic currents under a defined membrane voltage. This technically demanding method now has being automated by several groups (for a survey see Comley, 2003). One of the most sophisticated patch-clamp automates, the CytoPatch™, has been developed by CYTOCENTRICS. With the CytoPatch™ chip - a micro-structured glass chip, that replaces the conventional patch-pipette and all means for cell positioning (Fig. 1), - the same operation sequence as in conventional patch-clamping can be applied to suspended cells (Stett et al., 2003a). Due to the genuine micro fluidic concept for compound

application, it allows to apply the same protocols for whole-cell analysis of voltage- and ligand-gated ion channel activity as the conventional whole-cell patch clamping does - with the same quality and information content. The first-generation CytoPatch™ automat operates up to 20 CytoPatch™ chips in parallel for testing suspended cells which express various ion channel types.

MEA Technology. The safety pharmacology issue triggered the need for higher-throughput methods enabling the electrophysiological screening of selected compounds against ion channel targets in their native environment and functional organic, cellular, and sub-cellular context. A valuable tool to record electrical activity of electrogenic cells with a high information content with respect to drug action in an intact cellular environment of cardiac myocyte cultures, brain slices, either acute or maintained in organotypic long-term culture, and explanted retinas is the MEA (microelectrode array) biosensor (Fig. 2). Using planar metallic microelectrodes (diameter 10 to 30 μm), it offers the unique possibility for non-invasive extracellular recording from as many as 60 sites simultaneously (Egert et al., 1998, Potter, 2001). MEAs are an ideal *in vitro* system to monitor both acute and chronic effects of drugs and toxins and to perform functional studies under physiological or induced patho-physiological conditions that mimic *in vivo* damages. By recording the electrical response of various locations on a tissue, a spatial map of drug effects at different sites can be generated, providing important clues about a drug's specificity.

Examples of MEA applications, that have been developed at the NMI for drug screening and discovery as well as safety pharmacology are the MEA CARDIO SENSOR for fast, easy and efficient monitoring of drug effects on cardiac action potential parameters and to assess the potential of QT interval prolongation; the MEA RETINA SENSOR for multisite recording of local electroretinograms *in vitro* to assess effects of pharmacological compounds and putative therapeutics, drug side effects as well as consequences of degeneration-related processes on retinal signalling; and the MEA BRAIN SENSOR that is suitable to study processes of neurite outgrowth, synaptogenesis and regeneration and the modulation of these processes by drugs (Stett et al., 2003b).

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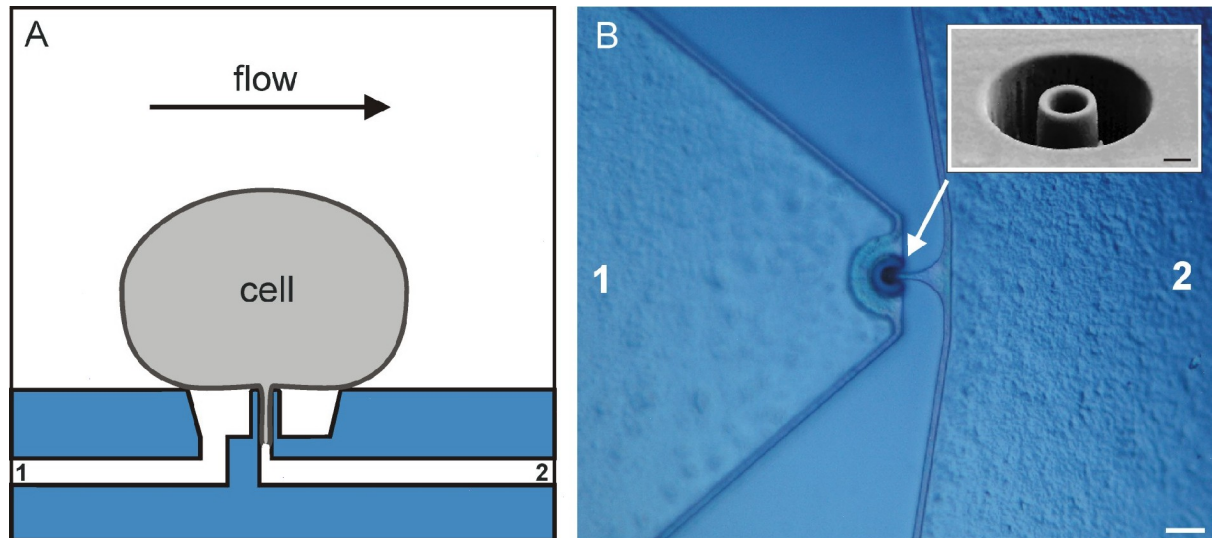


Fig. 1. CytoPatch™ chip for automated patch-clamping. (A) The planar electrode design mimics traditional patch clamping by using an outer channel (1) for cell positioning by suction and an inner opening (2) for contacting the cell. (B) Photograph of the micromachined patch-chip.

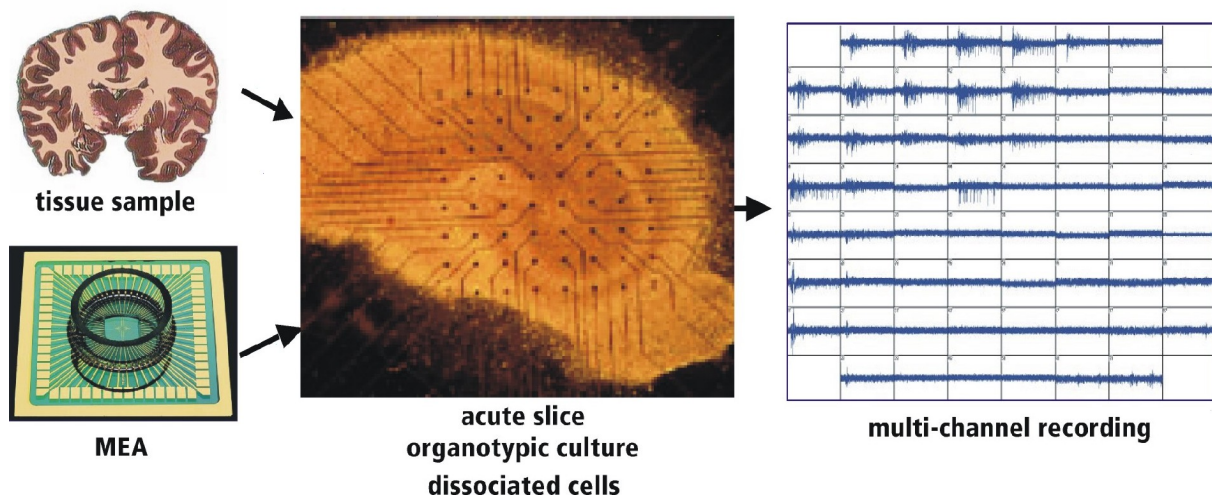


Fig. 2. MEA Biosensor. A brain slice is attached to the planar surface of the MEA (microelectrode array) with its substrate-integrated electrodes, which can be used both for multi-channel stimulation and recording.