Biosensors and Bioelectronics 26 (2011) 2376–2382

Contents lists available at ScienceDirect

Biosensors and Bioelectronics

journal homepage: www.elsevier.com/locate/bios

A cell-based impedance assay for monitoring transient receptor potential (TRP) ion channel activity

Oliver Pänke*, Winnie Weigel, Sabine Schmidt, Anja Steude, Andrea A. Robitzki

Centre for Biotechnology and Biomedicine (BBZ), University of Leipzig, Division of Molecular Biological-Biochemical Processing Technology, Deutscher Platz 5, D-04103 Leipzig, Germany

ARTICLE INFO

Article history:
Received 4 August 2010
Received in revised form 8 October 2010
Accepted 11 October 2010
Available online 16 October 2010

Keywords:
TRP cation channel
Impedance spectroscopy
Bioimpedance
Microelectrode array
Indium tin oxide
Chip-based screening assay
High content screening

ABSTRACT

Transient receptor potential (TRP) channels are non-selective ion channels permeable to cations including Na^+, Ca^{2+} and Mg^{2+}. They play a unique role as cellular sensors and are involved in many Ca^{2+}-mediated cell functions. Failure in channel gating can contribute to complex pathophysiological mechanisms. Dysfunctions of TRP channels cause diseases but are also involved in the progress of diseases. We present a novel method to analyse chemical compounds as potential activators or inhibitors of TRP channels to provide pharmaceutical tools to regulate channel activity for disease control. Compared to common methods such as patch clamp or Ca^{2+} imaging, the presented impedance assay is automatable, experimental less demanding and not restricted to Ca^{2+} flow. We have chosen TRPA1 from the TRPA ('ankyrin') family as a model channel which was stimulated by allyl isothiocyanate (AITC). HEK293 cells stably transfected with human TRPA1 cDNA were grown on microelectrode arrays. Confluent cell layers of high density were analysed. Impedance spectra of cell-covered and non-covered electrodes yielded a cell-specific signal at frequencies between 70 and 120 kHz. Therefore, 100 kHz was chosen to monitor TRPA1 activity thereupon. An average impedance decrease to about 70% of its original value was observed after application of 10 μM AITC indicating an increased conductance of the cell layer mediated by TRPA1. Transfected cells pretreated with 10 μM of inhibitor ruthenium red to prevent channel conductance, as well as control cells lacking TRPA1, showed no impedance changes upon AITC stimuli demonstrating the specificity of the novel impedance assay.

1. Introduction

The transient receptor potential (TRP) superfamily consists of a large number of cation channels. Most of them are permeable to both monovalent and divalent cations including Na^+, Ca^{2+} and Mg^{2+} (Pedersen et al., 2005; Ramsey et al., 2006). TRP cation channels are expressed in almost every tissue and cell type. Due to their central role in Ca^{2+}-mediated cell function and many homeostatic processes such as Ca^{2+} and Mg^{2+} reabsorption, failure in correct channel gating or permeation will likely contribute to complex pathophysiological mechanisms that cause human diseases (Inoue et al., 2006; Nilius, 2007; Kiselyov et al., 2007; Venkatachalam and Montell, 2007; Yamamoto et al., 2007; Watanabe et al., 2008). Here, we present a novel method based on impedance spectroscopy to screen chemical compounds as potential activators or inhibitors of TRP channels to provide pharmaceutical tools to regulate TRP channel activity for symptomatic and therapeutical disease control (Patapoutian et al., 2009). Compared to common methods used for the analysis of TRP channel gating such as patch clamp or optical techniques like Ca^{2+} imaging (Hill and Schaefer, 2007, 2009), the presented cell-based impedance assay employs microelectrode arrays (MEA) (Rahman et al., 2006; Wolf et al., 2008; Jahnke et al., 2009; Krinke et al., 2009) which is a promising approach of increasing interest for high throughput applications where a high degree of parallelisation is needed (Asphahani and Zhang, 2007; Yang and Bashir, 2008; Sadik et al., 2009). The presented assay is automatable, less demanding in respect to the experimental abilities of the conducting technician (patch clamp) and not restricted to the flow of Ca^{2+} ions (Ca^{2+} imaging).

Cell-based impedance spectroscopy is stimulated by the pioneered work of Giaever and Keese (1984) and allows a comprehensive electrical characterisation of adherent cell layers on microelectrodes. It is qualified to detect morphological changes (Giaever and Keese, 1993; Keese and Giaever, 1994), cell attachment and spreading (Mitra et al., 1991; Asphahani et al., 2008) as well as cell motility and migration (Giaever and Keese, 1991; Lo et al., 1993; Wegener et al., 2000) in real-time without any interfering labelling techniques. Cell-based impedance spectroscopy is commonly used to detect morphological changes within an united cell structure as a response to an external stimulus such as viral

* Corresponding author. Tel.: +49 0341 97 31242; fax: +49 0341 97 31249.
E-mail addresses: oliver.paenke@bbz.uni-leipzig.de, opaenke@uos.de (O. Pänke).

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doi:10.1016/j.bios.2010.10.015

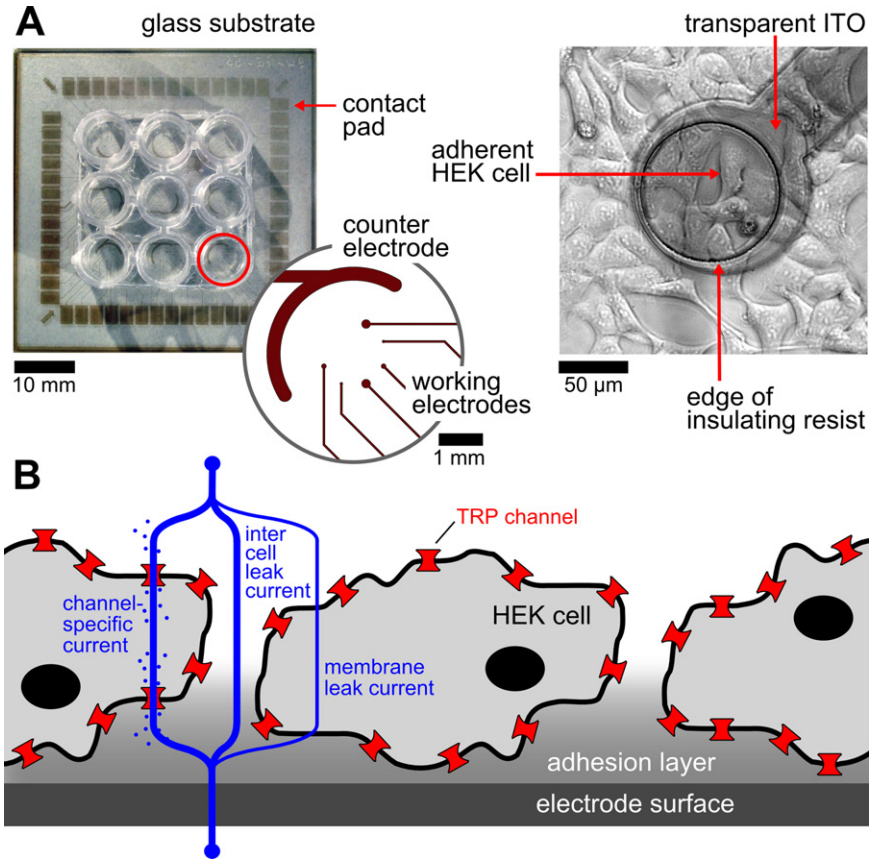

Fig. 1. (A) Microelectrode array (MEA) used for detection of TRPA1 ion channel conductance. The custom-made MEA 9W6E has nine wells, each containing three pairs of ITO electrodes with diameters of 50, 100 and 200 µm, which are transparent and allow microscopic imaging of the adherent cell layer. The distance between adjacent electrodes is approximately 570 µm and the ring-shaped counter electrode has an area of 1.8 mm^2. (B) Scheme of adherent HEK293 cells on the electrode surface and possible current paths defining the measured impedance. For better cell adherence a collagen adhesion support was applied (not to scale).

infection (Niikura et al., 2004; Mccoy and Wang, 2005; Campbell et al., 2007) or chemical compounds of cytotoxic (Tiruppathi et al., 1992; Keese et al., 1998; Ko et al., 1998; Arndt et al., 2004; Xing et al., 2005; Krinke et al., 2009; Opp et al., 2009) or pharmacological (Wolf et al., 2008; Jahnke et al., 2009) relevance. Such stimuli primarily affect the electrical conductance of the intercellular space or the cell–electrode interface rather than changes of cell membrane conductance or capacitance. The applied microelectrodes are often non-transparent and do not allow microscopic imaging of the adherent cells. Ion channel characterisation using cell-based impedance spectroscopy is rarely found in the literature, although ion channels located in cell membranes have crucial roles in physiology and pathophysiology and are important targets for drug discovery (Curran, 1998; Wissenbach et al., 2004; Okuhara et al., 2007; Jegla et al., 2009; Patapoutian et al., 2009; Mathie, 2010; Nassini et al., 2010). Impedimetric single cell analysis of ion channel activity in bovine chromaffin cells might serve as a rare example (Han and Frazier, 2006) where the observed impedance changes could be directly attributed to a molecular compound within the cell membrane.

We have chosen human transient receptor potential ankyrin1 (TRPA1, also known as ANKTM1) from the TRPA ('ankyrin') family as model for the impedimetric analysis of ion channel conductance. TRPA1 is expressed in sensory neurons (Story et al., 2003) and has a central role in the pain response to endogenous inflammatory mediators (Kwan et al., 2006; Bautista et al., 2006) and to a diverse array of chemical irritants such as allyl isothiocyanate (AITC) (Jordt et al., 2004; Hinman et al., 2006; Macpherson et al., 2007). AITC is the main component of mustard oil and often used for TRPA1 activation in *in vitro* analyses. Channel-specific inhibitors are rare, less specific, targeting various members of the TRP channel family and require high working concentrations. However, inhibitor ruthenium red is known to suppress AITC-stimulated ion conductance of TRPA1 in *in vitro* analyses (Nagata et al., 2005).

Here, we demonstrate the monitoring of stimulated ion conductance of heterologously expressed TRPA1 using cell-based impedance spectroscopy which gauges changes of the TRPA1-mediated membrane conductance. HEK293 cells were used as expression system and cultured on microelectrode arrays (MEA) consisting of indium tin oxide (ITO) or platinum microelectrodes, respectively. The application of transparent ITO electrodes allowed direct optical control of cell growth and adherence on the electrode. Fig. 1A shows the geometry of the self-developed ITO-based MEA, the arrangement of the electrodes and adherent cells on a transparent ITO microelectrode. Under these conditions, the measured impedance of the cell-covered microelectrode is defined by three possible current paths (Fig. 1B, see also Giaever and Keese, 1991; Arndt et al., 2004): The channel-specific current depends on the ion gating status of the membrane-embedded channel protein, the inter cell leak current is influenced by morphological changes of the united cell layer, and finally to a minor extent, current can be passed unspecifically across the cell membrane. For reliable detection of TRPA1 activity, the fraction of the latter two needs to be sufficiently small and the TRPA1 channel-dependent current has to dominate the measured impedance behaviour.

The pursued goal of this work was to demonstrate that cell-based impedance spectroscopy is qualified to monitor the activity of specific ion channels within cell membranes which opens the opportunity for automated analysis of ion channel related pharmaceutical compounds. Additionally, we successfully tested the ability