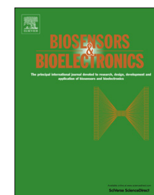
Biosensors and Bioelectronics 52 (2014) 89–97

Contents lists available at ScienceDirect

Biosensors and Bioelectronics

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



Label free sensing platform for amyloid fibrils effect on living cells

Mihaela Gheorghiu [a], Sorin David [a], Cristina Polonschii [a], Andreea Olaru [a], Szilveszter Gaspar [a], Ovidiu Bajenaru [b,c], Bogdan O. Popescu [d,e], Eugen Gheorghiu [a,*]

[a] International Centre of Biodynamics, 1 B Intrarea Portocalelor, 060101 Bucharest 6, Romania
[b] Department of Neurology, Neurosurgery and Psychiatry, University of Medicine and Pharmacy "Carol Davila", Bucharest, Romania
[c] Clinical Department of Neurology, University Emergency Hospital, 169 Splaiul Independentei, RO-050098, Bucharest 5, Romania
[d] Laboratory of Molecular Medicine, 'Victor Babeş' National Institute of Pathology, 99-101 Spl. Independentei, 050096 Bucharest 5, Romania
[e] Department of Neurology, Colentina Clinical Hospital – CDPC, School of Medicine, "Carol Davila" University of Medicine and Pharmacy, 19-21 Sos. Stefan cel Mare, 020125 Bucharest 2, Romania

A R T I C L E I N F O

Article history:
Received 22 July 2013
Accepted 17 August 2013
Available online 26 August 2013

Keywords:
Surface plasmon resonance
Electrical impedance spectroscopy
Label-free multiparametric real-time monitoring
Amyloid beta fibrils
Cellular dynamics
Cellular platform

A B S T R A C T

This study presents a multiparametric label-free analysis gathering surface plasmon resonance (SPR) and electrical impedance spectroscopy (EIS) for monitoring the progress of a model epithelial cell culture (Madin Darbey Canine Kidney – MDCK) exposed to a peptide with high bio-medical relevance, amyloid β (Aβ_{42}). The approach surpasses the limitations in using the SPR angle for analyzing confluent cell monolayers and proposes a novel quantitative analysis of the SPR dip combined with advanced EIS as a tool for dynamic cell assessment.

Long, up to 48 h time series of EIS and SPR data reveal a biphasic cellular response upon Aβ_{42} exposure corresponding to changes in cell-substrate adherence, cell–cell tightening or cytoskeletal remodeling.

The equivalent circuit used for fitting the EIS spectra provided substantiation of SPR analysis on the progress of cell adhesion as well as insight on dynamics of cell–cell junction.

Complementary endpoint assays: western blot analysis and atomic force microscopy experiments have been performed for validation.

The proposed label free sensing of nonlethal effect of model amyloid protein at cellular level provides enhanced resolution on cell-surface and cell–cell interactions modulated by membrane related protein apparatus, applicable as well to other adherent cell types and amyloid compounds.

© 2013 Elsevier B.V. All rights reserved.

1. Introduction

Multi-parametric label free cellular platforms hold substantial potential in dynamic assessment of subtle, nonlethal effects at cellular level, in a substantial step forward against endpoint analyses. An application area of major analytical and bio-medical relevance concerns evaluation of the direct interaction of specific compounds with different cell compartments, as an avenue to explore cellular processes in conjunction with toxicity effects.

One such example is provided by amyloid β (Aβ) peptides, compounds considered pivotal in Alzheimer's disease (AD) pathogenesis that showed a complex repertoire of effects at various levels within cellular models. Depending on the concentration, molecular structure and fibrilar state, Aβ peptides have been proved to exert various toxic cellular effects via disturbance of the structure and function of cell membranes (Williams and Serpell, 2011; Capone et al., 2009; Verdier et al., 2004), stress fiber formation, disruption and aggregation of actin filaments and cellular gap formation (Nagababu et al., 2009), increased trans-epithelial transport of large molecules (Deli et al., 2010; Gonzalez-Velasquez et al., 2008; Marco and Skaper, 2006) in conjunction with altered tight junctions (TJ) protein expression. This complex repertoire of, sometimes contradictory effects has been assessed using mostly end-point, individual analyses on various cell types or barrier models, avoiding standardization.

In this context, we report on a multiparametric biosensing platform combining Surface Plasmon Resonance (SPR) and electrical impedance spectroscopy (EIS) label-free assays to reveal cellular changes within a fully developed cell monolayer upon exposure to amyloid β fibrils. While the combination of these two techniques is not new (Terrettaz et al., 1993), this is, to the best of our knowledge, the first study to correlate the cell-surface dynamics assessed by SPR with electrical cellular and intercellular parameters provided by multifrequency EIS analysis for fully developed cell layers.

SPR matured into a powerful, well established analytic tool in biosensing, whereas SPR exploitation in biological cell monitoring has been only recently acknowledged. Reported applications range

* Corresponding author. Tel.: +402 131 043 54; fax: +402 131 043 61.
E-mail address: egheorghiu@biodyn.ro (E. Gheorghiu).

0956-5663/$ - see front matter © 2013 Elsevier B.V. All rights reserved.
http://dx.doi.org/10.1016/j.bios.2013.08.028

from SPR-based measurements of volume changes in adherent cells (Vala et al., 2012), cell activation (Cuerrier et al., 2008) to virus mimetic interactions (Chabot et al., 2009). The measurements are label-free, noninvasive and provide a relevant time resolution cell dynamics and rely on sensitive detection of changes in refractive index of cell monolayer due to cell-surface contacts and protein/lipid changes. Related optical biosensor technologies (Fang et al., 2007) are used to record integrated cellular responses rather than individual components of signaling pathways via label free monitoring of the redistribution of intracellular constituents triggered upon receptor activation (Schroder et al., 2010) as well as cell–cell and cell-surface interactions (Yashunsky et al., 2010).

SPR imaging approaches on whole cells (Yanase et al., 2010; Yanase et al., 2012) showed exquisite sensitivity of SPR assay on changes associated with plasma membrane, cell spreading, protein translocation and membrane potential, further confirmed through plasmon based impedance microscopy technique, pioneered by Tao group (Foley et al., 2008; Wang et al., 2011).

Complementarily, electrical impedance spectroscopy, a well established technique in cell assessment, provides noninvasive, real time monitoring capabilities of the electrical and morphological parameters of cell monolayers. Key contributors to the impedance measurements are changes in cell-substrate adherence, changes in cell shape and volume (Gheorghiu, 1996) and changes in cell–cell interactions (Gheorghiu et al., 2002; Sandu et al., 2010). These factors individually or collectively affect the flow of extracellular and trans-cellular current, influencing the magnitude and characteristics of the signal measured. As such, Electrode Cell substrate Impedance Sensing (ECIS) (Giaever and Keese, 1991), a validated marker for barrier integrity in real time (Jepson, 2003), enables label free monitoring of the interactions between cells and the substrate, study of cell properties such as attachment, spreading, motility, growth and proliferation (Asphahani et al., 2008; Ghenim et al., 2010; Giaever and Keese, 1991; Han et al., 2007; Hong et al., 2011) and cellular state (Arndt et al., 2004; Gheorghiu et al., 1999). The method enables real-time analyses to monitor cellular responses to chemical, physical, and biological stimuli (Hong et al., 2011). Interestingly, ECIS type assays, based on cellular index, have also been used to follow cellular response to G-protein coupled receptors activation in real time, revealing compound (Abassi et al., 2009), cell line (Peters and Scott, 2009) and signaling cascade (Kammermann et al., 2011) characteristic impedance-based time-dependent cell response profiles.

While a certain degree of convergence is evident for SPR and EIS data in particular for cell-surface interaction, as both techniques are sensitive to surface coverage, the combination of the two techniques holds potential in providing additional insight into cellular processes at various levels within cell structure: SPR regarding intracellular refractive index shifts, whereas EIS will provide data on cell–cell interactions with special focus on cell barrier properties. As such this combined platform is particularly applicable for assessment of the effect of target compounds with complex biological interactions at various levels within cell structure (such as Aβ) to yield insight into the fibril associated effect on a model cell culture.

As model compound, we choose the $A\beta_{1-42}$ fragment, one of the predominant form of Aβ found in brains of AD patients, based on its well established cytotoxic profile (Klein et al., 2004) and documented self-association propensity (Jarrett et al., 1993). Interaction between this amyloid protein and cellular membranes, as well as the associated cellular effect is assumed to be important both in disease onset and propagation.

MDCK (Madin Darbey Canine Kidney cells), provide a well characterized model of polarized epithelial cells, a suitable and robust cell model for investigation of Aβ effect on both "naive" and specialized (e.g. brain) barriers, easy to grow and highly relevant for elucidating whether Aβ related alterations are univocally achieved in the **in situ** microenvironment of the Blood Brain Barrier (BBB) or can be exerted in an externalized environment as well. MDCK cells were applied to the study of polarization of amyloid precursor peptide trafficking and sorting, as models for similar events that go on in neuronal cells (Haass et al., 1994, 1995); (Capell et al., 2002) and as in vitro model for the transport of Aβ across the BBB, (Nazer et al., 2008). Being of renal origin, MDCK cells provide an added bonus given the documented: (i) greater renal impairment for AD patients than for cognitively normal controls (Kerr et al., 2009), (ii) the influence on amyloid homeostasis of reduced renal clearance of peripheral Aβ, as well as (iii) the recently drawn epithelial parallels in neuronal adhesion control (Famulski and Solecki, 2012).

The proposed label free sensing of nonlethal effect of model amyloid protein at cellular level enables enhanced resolution on cell-surface and cell–cell interactions modulated by membrane related protein apparatus, applicable as well to other adherent cell types. To this purpose, the following advancements have been pursued:

- combined EIS/SPR platform for real time, noininvasive assessment of the multiparametric changes of fully developed cell monolayers exposed to Aβ fibrils;
- monitoring biological effects at the level of cell-substrate adherence, cell interior and cell–cell junctions, using a novel SPR dip analysis and multichannel, multifrequency EIS analysis of cell monolayers based on a realistic equivalent circuit;
- cross validation of cell substrate interaction dynamics provided by SPR and EIS data.

Endpoint Western Blot (WB) and Atomic Force Microscopy (AFM) analyses complete, with molecular and high resolution morphological snapshots, the multi-parametric insight of the Aβ fibrils effect on cell monolayers.

2. Materials and methods

2.1. Chemicals

2.1.1. $A\beta_{42}$ fibril formation

Synthetic $A\beta_{42}$ (Sigma, A9810) was reconstituted in cell media as previously reported (Cedazo-Minguez et al. 2003) to form (proto)fibrils. Briefly, peptides were suspended in serum free cell medium (Dulbecco's modified Eagle's medium, DMEM pH 7.4) at a concentration of 10 μM and incubated at 37 °C, 5% CO_2 for 48 h, with occasional stirring. Cell treatment, was performed on cells at 90% confluence, at a concentration of 5 μM.

2.1.1.1. Cell cultures. MDCK I cells (ECACC, cat. no. 00062106), were grown on in DMEM/F12 medium (Invitrogen) supplemented with 10% fetal calf serum, 2 mM L-glutamine, 100 U/mL penicillin, and 100 μg/mL streptomycin at 37 °C in a 5% CO_2 humidified incubator. Passages were performed at 80% confluence and cells were seeded at 10^5 cells/cm^2 on the measurement chips. Environmental control is ensured during subsequent cellular assays within Sanyo MCO-20AIC CO_2 Cell Culture Incubator.

2.1.2. Biomimetic lipid membranes

It was shown that zwitterionic supported phospholipid bilayers selectively bind Aβ aggregates but not the monomeric form (Kotarek and Moss, 2010). To check for the effective Aβ concentration that would potentially bind to the cell membrane and alter

Download English Version:

https://daneshyari.com/en/article/866756

Download Persian Version:

https://daneshyari.com/article/866756

Daneshyari.com