Dynamic assessment of Amyloid oligomers – cell membrane interaction by advanced impedance spectroscopy

M Gheorghiu, S David, C Polonschii, D Bratu and E Gheorghiu.
International Centre of Biodynamics, Intr. Portocalelor 1B, 060101 Bucharest, Romania, www.biodyn.ro
e-mail: mgheorghiu@biodyn.ro

Abstract. The amyloid β (Aβ) peptides are believed to be pivotal in Alzheimer’s disease (AD) pathogenesis and onset of vascular dysfunction. Recent studies indicate that Aβ treatment influences the expression of tight junction protein complexes, stress fibre formation, disruption and aggregation of actin filaments and cellular gap formation.
Aiming for functional characterization of model cells upon Aβ treatment, we deployed an advanced Electric Cell-substrate Impedance Sensing for monitoring cell evolution. A precision Impedance Analyzer with a multiplexing module developed in house was used for recording individual electrode sets in the 40 Hz - 100 KHz frequency range. In a step forward from the classical ECIS assays, we report on a novel data analysis algorithm that enables access to cellular and paracellular electrical parameters and cell surface interaction with fully developed cell monolayers.
The evolution of the impedance at selected frequencies provides evidence for a dual effect of Aβ exposure, at both paracellular permeability and cell adherence level, with intricate dynamics that open up new perspectives on Aβ oligomers – cell membrane interaction.
Validation of electrical impedance assays of the amyloid fibrils effect on cell membrane structure is achieved by both AFM analysis and Surface Plasmon Resonance studies.
The capabilities of this noninvasive, real time platform for cell analysis in a wider applicative context are outlined.

1. Introduction
The amyloid β (Aβ) peptides are believed to be pivotal in Alzheimer’s disease (AD) pathogenesis and onset of vascular dysfunction. Recent studies [1],[2] indicate that Aβ treatment influences the expression of tight junction protein complexes, stress fiber formation, disruption and aggregation of actin filaments and cellular gap formation. Nevertheless, the exact mechanisms involved in these effects are still a matter of debate due to the variability in the investigation methods used, the type of cells analyzed and the nature of Aβ compound (type, oligomer/fibril form).

In a “reductionist approach” we focus on Aβ effect on cellular barrier properties outside the complex microenvironment of the brain, on selected renal cells incorporated in an ECIS type device developed recently in ICB.
The ECIS devices [3] enable noninvasive, continuous assessment of cell attachment, spreading and proliferation and hence can be a powerful replacement for the traditional Transepithelial Electrical Resistance (TEER) determinations, a validated marker of barrier integrity in real time, for assessment of cytotoxic effects. They are based on AC impedance measurements using weak, noninvasive signals.
(up to 50 mV) in the frequency range: 1 Hz - 10 MHz. Attachment and spreading of cells on the electrode surface change the impedance enabling direct inference of the morphological information on the attached cells. Multifrequency impedance monitoring, as opposed to single frequency assays, is a prerequisite for discerning between the various processes involved in cell response and enable data deconvolution by modeling.

The platform enables dynamic assessment of amyloid oligomers – cell membrane interaction by advanced impedance spectroscopy and provides enhanced resolution on cell-surface and cell-cell interactions modulated by membrane related protein apparatus, applicable as well to other adherent cell types.

2. Experimental section
The measurement system is equipped with 4 pairs of planar, circular electrodes, manufactured by photolithography and metal deposition processes on glass slides, placed as bottom of the cell growth chambers.

For parallel control and test experiments we used a measurement chamber with two identical compartments [4]. An amount of $10^5$ cells were seeded into each compartment of the measuring chamber and left to grow (standard growth conditions) into a subconfluent layer (2 days). Upon treatment, the culture medium was removed from both compartments and replaced with fresh, serum free medium (control) and 5 µM “aged” Aβ42 in serum free medium (Aβ). The ageing process, 48 h at 37 °C, 5% CO2, determines forced aggregation of Aβ42 monomers into oligomers and (proto)fibrils.

A 4294 A Precision Impedance Analyzer, Agilent Technologies Ltd., Japan, with a multiplexing module developed in house was used for recording the four individual electrode sets. An AC potential was used with zero bias, 50mV pp amplitude, 40 Hz - 100 KHz frequency range (100 frequency points with logarithmic distribution).

Data analysis was performed based on a modified cell index $V_N$ given by Eq. 1:

$$V_N(t) = \frac{V(f_{rl})}{V(f_{rh})} (t) \frac{V(f_{rl})}{V(f_{rh})} (t_0)$$

where $f_{rl}$ and $f_{rh}$ correspond to measurement points in the low and high frequency domain, respectively, and $t(0)$ corresponds to a reference moment chosen after cell culture stabilization.

This normalization eliminates environmental differences (e.g. temperature, growth medium changes) while providing a broad sensitivity response.

3. Results
Based on Eq.1, the impedance spectra covering the frequency range 40Hz – 100 kHz are normalized, for each frequency point (by deriving the relative variation), versus real and imaginary impedance spectra at the beginning of the experiment or upon Aβ42 addition. Figure 1 shows the distinct dynamics of the cell layer under Aβ42 exposure and in controlled conditions ($f_{rl}=100$ Hz and $f_{rh}=10$ kHz).

![Figure 1](image-url)

**Figure 1.** Time evolutions of Normalized impedance Real and Imaginary parts for control (black) and Aβ42 exposed cells (red); arrow indicates Aβ addition or medium exchange.
In the experimental time frame, there are no cell-cell junctional complexes formed, yet, distinct dynamics are evident for both cell-surface contacts and cell growth (as revealed in different regions of the frequency spectrum), figure 2.

![Figure 2. Effect of Aβ42 exposure on MDCK cells as revealed by different frequency regions considered in the estimation of \( V_N \) (\( f_{rl}=100\,\text{Hz} \) and \( f_{rh}=20\,\text{kHz} \), red; \( f_{rl}=2.5\,\text{kHz} \) and \( f_{rh}=100\,\text{kHz} \), blue); the arrow indicates Aβ addition](image)

The multiphasic dynamics evidenced in Figure 2 upon Aβ42 addition can be related to cell-surface tightening or protein mobilization toward the membrane (above 10 h) but discrimination between these processes cannot be made.

When coping with the whole frequency domain, the experimental data can be fitted with equivalent electrical circuits, that associate distinct functional entities in the biological probe (e.g. cell monolayer, paracellular space, cell – surface and cell-to-cell contacts) with abstract representations (e.g. constant phase elements, resistors and capacitors). Nevertheless, such a circuit has to be optimized for increased robustness and rendering capability to fit large time series of impedance data. Therefore, we considered a circuit depicted in figure 3, comprising a resistor for the subcellular and the bulk media, capacitor for the electrode system and a constant phase element, CPE for the cellular layer (characterized by 2 parameters: \( CPE_T \) and \( CPE_P \)) according to the following equation:

\[
Z_{CPE} = \frac{1}{CPE_P \cdot (i \cdot \omega)^{CPE_P}}.
\]  

(2)

![Figure 3. Equivalent circuit](image)

The parameter \( CPE_P \) is such that if \( CPE_P = 1 \), the impedance of a CPE is that of an ideal capacitor, and when \( CPE_P = 0 \), the CPE is a pure resistor. The frequency dependence of this element has been ascribed to a wide variety of effects such as surface roughness and heterogeneity, nonuniform current distribution, and distribution of relaxation times. Given the possible Aβ42 effect on the permeability of cell junctions, a change of \( R_2 \) is expected (reflecting changes in the adherence to neighbour cells) as usually evidenced by classical TEER. Nevertheless, a more general toxic effect (Aβ exposure induces generation of toxic reactive oxygen species [5]), should influence also cell adherence points to the substrate and overall cell growth. An increase of \( CPE_T \) should occur due to cells rounding up and displaying a larger surface area, as well as a decrease of \( CPE_P \) (since the heterogeneity of the surface decreases). Noteworthy, we have recently revealed a similar effect when reactive species are generated upon mechanical and chemical stimulation [4]. The evolution of fitted data (Figure 4) confirms this latter effect of Aβ42 exposure cell adherence level: better cell-surface attachment is evident after 10 h of Aβ42 exposure (see also figure 4 inset).
Validation of electrical impedance assays of the amyloid fibrils effect on cell membrane structure can be achieved by both AFM analysis and Surface Plasmon Resonance studies [6].

4. Conclusions
Based on an improved ECIS approach, we reveal the complex, non-monotonous cell behavior in response to Aβ exposure, otherwise difficult to be assessed through end-point measurements. The proposed cellular platform is ideally positioned for more general, label free, cytotoxic evaluation of nonlethal effect of complex mixtures or unknown compounds as it noninvasively provides continuous assessment of: cell–surface and cell-cell interaction, proliferation as well as intracellular changes.

5. Acknowledgment
We gratefully acknowledge the financial support of grant BIOSCOPE (PN-II-ID-PCCE, Contract No 11/2012).

References
[1] Marco S and Skaper S D 2006 Neurosci Lett. 401(3), 219-224.
[2] Nagababu E, Usatyuk P V, Enika D, Natarajan V and Rifkind J M 2009 J. Alzheimers Dis. 17(4), 845-854.
[3] Giaever I and Keese C R 1993 Nature 366, 591–592
[4] Gaspar S, David S, Polonschii C, Marcu I, Gheorghiu M and Gheorghiu E 2012 Anal. Chim. Acta 713 115-120
[5] Wang H, Ma J, Tan Y, Wang Z, Sheng C, Chen S and Ding J 2010 J. Alzheimers Dis. 21 597-610
[6] Gheorghiu M, David S, Polonschii C, Olaru A, Bajenaru O, Gaspar S, Popescu B O and Gheorghiu E submitted 2013 Biosens. Bioelectron.