Study of the dopamine effect into cell solutions by impedance analysis

G Paivana¹, T Apostolou¹, G Kaltsas² and S Kintzios¹
¹Department of Biotechnology, Agricultural University of Athens, Greece
²Department of Electronic Engineering, Technological Educational Institute of Athens, Greece
*Corresponding author: georpaiv@gmail.com

Abstract. Electrochemical Impedance Spectroscopy (EIS) has become a technique that is frequently used for biological assays. Impedance is defined as a complex – valued generalization of resistance and varies depending on its use per application field. In health sciences, bioimpedance is widely used as non-invasive and low cost alternative in many medical areas that provides valuable information about health status. This work focuses on assessing the effects of a bioactive substance applied to immobilized cells. Dopamine was used as a stimulant in order to implement impedance analysis with a specific type of cells. Dopamine constitutes one of the most important catecholamine neurotransmitters in both the mammalian central and peripheral nervous systems. The main purpose is to extract calibration curves at different frequencies with known dopamine concentrations in order to describe the behavior of cells applied to dopamine using an impedance measurement device. For comparison purposes, non-immobilized cells were tested for the same dopamine concentrations.

1. Introduction
Electrical impedance spectroscopy (EIS) [1], as a non-invasive, real-time method [2], is a technique where the electrical impedance of living cells is measured to characterize various types of cell or to discriminate pathological cells from normal ones based on the electrophysiological properties of cells in the frequency domain [1, 3]. The technique involves application of a small alternating current (AC) signal over a wide frequency range on the electrochemical cell and measuring current response. Based on the cells electrical response over a particular frequency range [1] the impedance of the biocompatible electrodes with cells, the changes in their cell death [4] or toxicity [5] can be detected [2]. Impedance measurements have been demonstrated as a powerful tool for the real-time study of complex biological systems both in vivo [6] and in vitro [7-10] by establishing a correlation between the electrical measurements and the biological phenomena [11).

Dopamine ((3, 4-dihydroxyphenyl) ethylamine, DA) is one of the important catecholamine neurotransmitters in both the mammalian central and peripheral nervous systems [12] and plays a significant role in the functioning of central nervous, renal, and hormonal systems [13].

In the brain, this catecholamine functions as a neurotransmitter, activating the five types of dopamine receptors - D1, D2, D3, D4 and D5, and their variants. In particular, the pharmacological modification of the responsiveness of the different dopamine receptors (D1-D5) and their subtypes is the basis of the treatment, as well as the side effects of major diseases and pathological conditions, such as schizophrenia, Parkinson’s disease, Tourette syndrome, drug addiction, and hyperprolactinemia [14].

Over the last decade, mammalian cell-based assays have grown considerably in significance in clinical analytical science, offering the combined advantage of high-sensitivity and non-invasive or
minimally invasive monitoring. Even more important is their multipurpose catalytic capacity, enabling cellular biorecognition elements to provide information about the actual effects of target analytes, even if these effects represent the synergistic or cumulative response of the interaction of said analyte with different receptor subtypes [15, 16]. In addition, assay principles based on measuring cellular bioelectric properties (such as impedance or membrane potential) are quite popular, due to their speed, quantification and relative ease of use [17].

Im immobilization of the cells onto the transducer’s surface simplify operation and efficiently improve storage and operational stability. Immobilization procedures applied for this purpose should preserve cell’s functionality and viability, as well as to ensure efficient access of analyte molecules to the cells and of product molecules, generated by the intracellular enzymatic activity, to electrode’s surface.

In the present study we report a novel methodological concept for the development of functional assays for the in vitro interaction of DA with N2a mouse neuroblastoma cells. The novel approach is characterized by considerable speed (3 minutes), sensitivity (measuring the response of cells against DA concentrations as low as 1 μM) and reproducibility.

2. Experimental details
Chemicals: Murine neuroblastoma (N2a) cell cultures were originally provided from LGC Promochem (UK) and subcultured in Dulbecco’s medium with 10% fetal bovine serum (FBS), 1U μg⁻¹ antibiotics (penicillin/streptomycin) and 2mM L-glutamine which were provided from Invitrogen (CA, USA). All other reagents were purchased from Sigma-Aldrich (Taufkirchen, Germany).

Cells preparation/immobilization: Bactoagar gel was used as the 3D immobilization matrix of choice. The required mass was weighted and diluted in water and then put for sterilization. The final concentration was 1.2%. Afterwards, the gel was heated at 37°C in order to use it for cell immobilization. A cell culture was carried out in order to prepare the cells for impedance measurements. Before each assay, cells were detached from the culture and concentrated by centrifugation (2 min, 1200 rpm, 25°C). After the process of culture was completed, 50.000 N2a cells were mixed with 50 uL of the Bactoagar gel (1% final concentration) and poured together in the well of the electrode. By adding 40uL nutrient in the well, the resulting volume was 90uL. DA solutions in double distilled water were prepared freshly on the day of each assay. For measurements, DA was applied in different concentrations (1μM, 10μM, 100μM, 1000μM). In each case, either immobilized/non-immobilized cells solutions were placed in the electrode system in order to measure the impedance.

Experimental setup: In this work, the handheld LCR meter U1733C (Keysight Technologies, location) was used. Each experimental procedure was evaluated in three pre-selected frequencies given from the device; 1KHz/10KHz/100KHz. The duration of each measurement run was three minutes, therefore given a measurement frequency of 1Hz the total measurement values were 180 for each run.

3. Results and Discussion
This work focuses on assessing the effects of DA applied to immobilized/non-immobilized cells. Dopamine was used as a stimulant in order to implement impedance analysis with a specific type of cells. The main purpose is to extract calibration curves at different frequencies with known dopamine concentrations in order to describe the behavior of cells using an impedance measurement device.
The results of the impedimetric assay demonstrated a clear pattern of response depending on the range of DA concentrations. As it can be easily observed in figure 1, in all cases the impedance value drops with increasing concentrations.

![Figure 1: N2a cells response at different frequencies with increasing DA concentrations](image)

As showed from previous work [17] DA concentration at the range of 1 – 1000 uM cause activation of D2-like receptors, associated with inhibition of cAMP accumulation and increase of outward potassium currents, leading to cell membrane hyperpolarization.

In order to mimic in vivo conditions cells were immobilized in Bactoagar gel, as described in previous section and treated with DA solutions at different concentrations as seen in figure 2.

![Figure 2: Immobilized N2a cells response at different frequencies with increasing DA concentrations](image)
Immobilized cells, in particular cells entrapped in a 3D Bactoagar cell, represent more accurately (though not perfectly) the actual environment in vivo, under in vitro simulative conditions. The results of using an immobilization method revealed an almost same pattern as previous, leading to cell membrane hyperpolarization, showing the prospective of using our system in experiments that mimics accurately the cells conditions.

4. Conclusion
Impedance measurement is known as a method widely accepted as a non-invasive and easy-to-use as it provides valuable information about the sample tested. Biological cell impedance properties are usually used to investigate cells themselves or the body’s healthy status. In this work, an impedance analysis was evaluated on N2a cells applied to different DA concentrations, whereas the innovative aspect, compared to all previous reports, was the investigation of the 3D cell immobilization. The observed results have shown that the impedance observed under cell immobilization regimes plays a significant role as it describes the electrical response of the cell medium when frequency is applied. This information can be useful for medicine evaluation that interfere dopamine’s absorption from cells, for instance Parkinson disease, schizophrenia, etc.

Further improvements in our methodological approach include the study of the effect of the electrode material (e.g. gold vs silver or carbon), different immobilization materials as well as the use of a wider frequency range especially in lower frequencies. We envision that when optimized the immobilized cell impedimetric system will be applied to the analysis of the several types of target analytes with bioactive properties.

References
[1] Hong J L, Lan K C and Jang L S 2012 Sens. Act. B 173 927-934.
[2] Jun H S, Dao L T, Pyun J C and Cho S 2013 Enzy. Micro. Tech. 53 302-306.
[3] Rigaud B, Morucci J P and Chauveau N 1996 Crit. Re. Bio. Eng. 24 257-351.
[4] Patel P and Markx G H 2008 Dielectric measurement of cell death Enz. Micro. Tech. 4 463-70.
[5] Xiao C and Luong J H T 2005 Toxi. Ap. Pharm. 206 102-12.
[6] Weijenborg P W, Rohof W O, Akkermans L M, Verheij J, Smout A J and Bredenoord A J 2013 J. Neuro. Mot. 25 574-e458.
[7] Giaever I and Keese C R 1984 Proc. Natl. Acad. Sci. U S A 81 3761–64
[8] Daza P 2013 Sens. Actu. B 176 605-610.
[9] Kin F L, Wu M H, Liao P Y, Chen Y M and Pan T M 2012 Micro. Nano. 12 117-125.
[10] Lei K F, Wu M H, Hsu C W and Chen Y D 2014 Bios. Bioel. 51 16-21.
[11] Canali C, Heiskanen A, Muhammad H B, Høyum P, Pettersen F J, Hemmingsen M, Wolff A, Dufva M, Martinsen Ø G and Emnéus J 2015 Bios. Bioe. 63 72-79.
[12] Wang H S, Li T H, Jia W L and Xu H Y 2006 Bios. Bioe. 22 664-669.
[13] Song W 2010 J. Sol. St. Ele. 14 1909-14.
[14] Abraham A D, Neve K A and Lattal K M 2014 Neur. Lear. Mem. 108 65-77.
[15] Kintzios S 2007 Cell-based biosensors in clinical chemistry M. Re. Med. Ch. 7 1019-26.
[16] Banerjee P, Kintzios S and Prabhakarpandian B 2013 Toxins 5 2366-83.
[17] Apostolou T, Moschopoulou G, Kolotourou E and Kintzios S 2017 Talanta 170 69-73.