**ORIGINAL ARTICLE**

# CELLULAR IMPEDANCE MEASUREMENT – NOVEL METHOD FOR *IN VITRO* INVESTIGATION OF DRUG EFFICACY

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**Abstract**

There are a few literature data focusing on impedance measurement of melanocytes' cellular behaviour. We present herein the impedance measurement as a potential assay for studying the sensitivity of melanocytes, isolated from freshly excised tumours, to standard drugs. The methodology for investigating primary cells sensitivity to cytostatics like dacarbazine is presented. The cellular impedance method is a reliable and rapid method for monitoring dynamic cell behaviour following drug exposure and a future important step toward personalized therapy.

**Rezumat**

Actualmente, literatura medicală posedă puține date referitor la măsurarea comportamentului celular al melanocitelor prin impedanță. În cele ce urmează, prezentăm importanța măsurării impedanței pentru studiul sensibilității melanocitelor maligne, izolate din țesut tumoral proaspăt excizat, la medicamente utilizate standard în tratamentul melanomului; în acest sens, prezentăm un protocol de investigare a sensibilității celulare la citostatice de tipul dacarbazinei. Impedanța celulară se dovedește astfel a fi o metodă modernă, fezabilă, de încredere și rapidă pentru monitorizarea dinamică a comportamentului celular în urma expunerii la medicamente și reprezintă un pas important în direcția terapiei personalizate.

**Keywords**: cellular impedance, melanocytes, cytostatics, dacarbazine

## Introduction

Melanoma is a malignant tumour arising from melanocytes and represents the most severe oncologic diagnosis due to its metastatic potential. It is a real health problem as it is one of the most frequent tumour affecting adolescents and young adults, occurring in Caucasian females belonging to the 25 to 29 year-old age group [1-3].

Worldwide, the incidence rates of melanoma increased in the last years; it varies from 0.2 - 0.5 per 100,000 in India to 12 - 15 per 100,000 in Europe and 40 - 50 per 100,000 in Australia. The lifetime risk of developing melanoma varies from 1:100 in Europe to 1:25 in certain populations from Australia [2, 4].

Ultraviolet radiation exposure and the history of sunburn are the most important risk factors in developing melanoma. Studies showed that intermittent intense exposures to sun together with severe sunburn, especially during childhood, are associated with a higher risk for developing melanoma than continuous sun exposure. The etiologic role of sunlight is also supported by the higher incidence of melanoma in Caucasian patients residing closer to the equator. Indoor tanning is also an important risk factor for this pathology [5-7].

The prognosis of the disease depends on the stage of melanoma at diagnosis, the most important prognosis factors being tumour thickness, especially Breslow depth, ulceration and mitotic rate. Therefore, while patients with localized melanoma have a five year survival rate of over 95%, patients with distant metastases have a five year survival rate of approximately 16% and median survival rate of 6 to 8 months [1, 2, 8].

Several new agents, such as BRAF (oncogene that encodes for the protein known as serine/threonine-protein kinase B-Raf) inhibitors, MEK (mitogen-activated protein kinase kinase) inhibitors, anti PD1 (programmed cell death protein 1) antibodies or anti-CTLA-4 (cytotoxic T-lymphocyte-associated protein 4) antibody, have been recently approved in the treatment of advanced melanoma. However, the prognosis of patients with advanced melanoma remains poor, as resistance to all these new drugs was observed (8). Therefore, further studies are needed in order to find new treatment methods.

Melanoma is a highly aggressive skin malignancy with a rapidly increasing incidence rate. It often involves a complex treatment, and advanced forms can acquire therapy resistance [9-12].

The clinical course of the disease and the response to therapy can be influenced by numerous exogenous and endogenous factors, including immunomodulatory, inflammatory or neurogenic signals [13]. The investigation of various compounds effect on tumour cells and skin cells in general may reveal the molecular pathways of therapy resistance, and can contribute to a better choice of treatment [14, 15].

Using standard cell lines, although attractive, can lead to unexpected results and probably to important differences when investigating a drug in primary cell lines isolated from patients. The opportunity to use cells of human origin may improve the predictive value of assays in drug testing and came hand in hand with the development of innovative real-time monitoring of cellular behaviour.

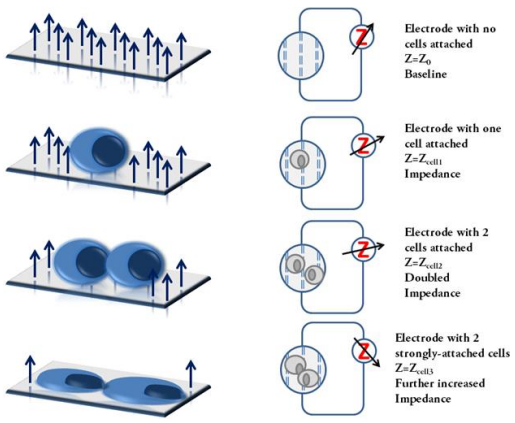


**Figure 1.**
Impedance measurement principle in real-time monitoring using xCELLigence system

Real-time monitoring of cell behaviour using impedance is a recent method that allows various studies in a single setup, by measuring electrical impedance across interdigitated micro-electrodes integrated on the bottom of tissue culture plates (Figure 1). xCELLigence real time cell analyser offers a label-free non-invasive manner long-term recording of cellular signals [16].

Considering all this aspects, the aim of our research was to evaluate the impedance of the primary melanocytes isolated from excised tumours that were confirmed as cutaneous melanoma. Primary cell cultures were used for establishing the efficacy pattern of a standard cytostatic, dacarbazine.

Our goal stands for obtaining a work flow for validating specific drug's efficacy in individual tumour cells as a mean to personalize therapy in melanoma.

## Materials and Methods

*Cell cultures and treatments*. Freshly excised cutaneous melanoma was subjected to a standard protocol. Briefly, the fragment was soaked in 70% ethanol for 30 seconds and rinsed in Hanks's balanced salt solution (HBSS). Fat and sub-cutaneous tissue was trimmed off and approximately 2 x 3 $mm^2$ pieces were cut. These pieces were transferred in dispase II. Pieces were incubated at 4°C for 15-18 hours in dispase. The tissue pieces were subsequently transferred in $Ca^{2+}$, $Mg^{2+}$ - free HBSS, and the epidermis was separated from the dermis. The collected epidermal sheets were transferred in 0.5% trypsin/versene solution and incubated at 37°C for 3-5 minutes. After vigorous shaking, cells were detached and cell suspensions were centrifuged for 5 minutes at 1,200 rpm at room temperature; the pellet was re-suspended in MCDB 153 medium (Sigma). Cells were seeded at 2 x $10^5$ cells/$cm^2$ in the tissue culture vessel and incubated at 37°C CO2/95% air for 48 - 72 hours. After several passages primary cell cultures were stable and were used for further *in vitro* experiments. For *in vitro* testing, standard dacarbazine was used in 0.25 mM concentration.

*Real-time monitoring of cell response using impedance technology*. Experiments were performed on E-16 plates (Roche, Penzberg Upper Bavaria, Germany, catalogue no.05469830001), compatible with RCTA-DP system (Roche Applied Science). The assay was performed on E16 plates, without any coating. Various cell concentrations were used, 10,000 – 1,250 cells/well to register cell index (CI). Cells were left to adhere for 2 h in the RTCA DP device at 37°C and 5% $CO_2$. Readings were collected at 1 minute intervals for 72 hours and the results reported as normalized CI to time just before dacarbazine addition. The assay system expresses impedance in arbitrary CI units. When late cellular response was investigated, cell media was replaced every 48 hours. Each situation was quadruplicated [16].

**Results and Discussion**

All E-16 plates were covered on the bottom with sensor electrodes capable of reading modification of electric resistivity induced by cell adhesion. The first 2 h upon seeding registers the actual adhesion of cells, while afterwards the impedance increment was registered as cells' proliferation. When investigating the proliferation capacity of primary melanocytes isolated from melanoma tumour we observed that there was a clear dependence of the CI upon the number of seeded cells (Figure 2). The normalized CI after 48h of cultivation showed that the best proliferative capacity was registered when 1,250 or 2,500 cells/well were seeded. When wells were seeded with higher cell concentration the proliferative capacity was hindered due to space confinement and nutrients exhaustion.

The choice to use only cells in basal conditions (steady state growth in normal tissue culture medium, without starvation and stimulation) for the assessment of effects displayed by pharmacological inhibitors is supported by Bartscht *et al.* [17]. This group showed that both stimulated cells and non-stimulated cells have a similar scattering behaviour related to inhibition. We have previously shown in glioblastoma primary cells that the behaviour of cells derived from the patients' tumours was slightly different when compared to a standard U87 cell line [18].

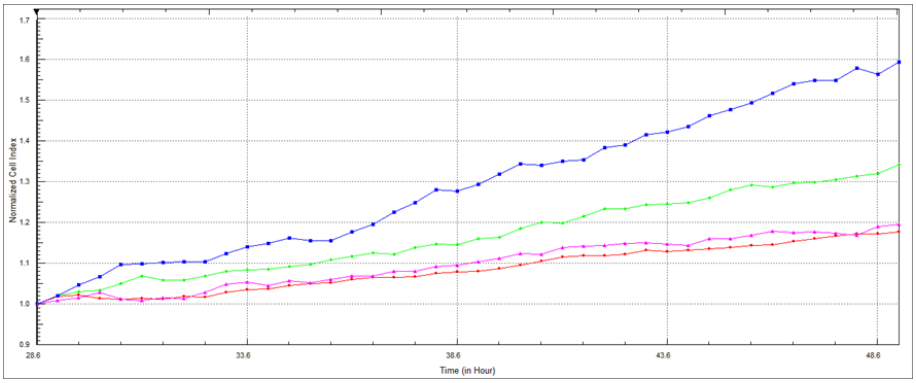


**Figure 2.**

Primary melanocytes isolated from melanoma tumour, seeded at 10,000 cells/well (red), 5,000 cells/well (purple), 2,500 cells/well (green) and 1,250 cells/well (blue) after 48 h of cultivation. The graph represents the normalized cell index

When investigating the effect of a standard cytostatic, like dacarbazine, on primary cell cultures (Figure 3) we can evaluate that the CI of treated neoplastic melanocytes decreases to 75%. Knowing that the dacarbazine concentration is a low one, we can ascertain that the method is sensitive for evaluating the efficacy of the tested drug.

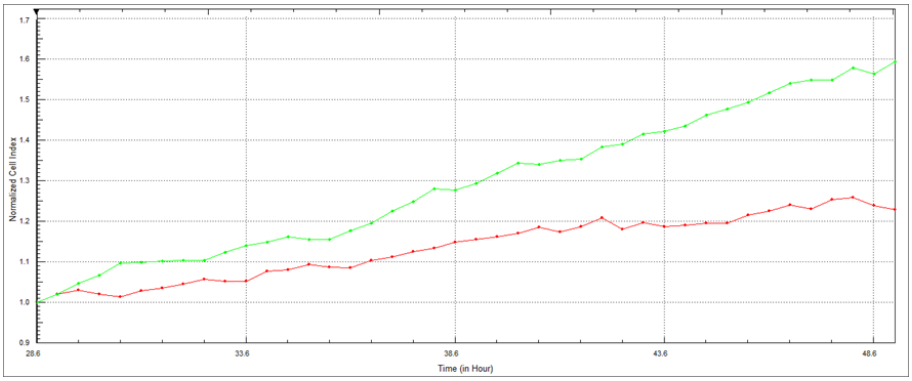


**Figure 3.**

Primary melanocites isolated from melanoma tumour, seeded at 1,250 cells/well (green) in the presence of dacarbazine 0.25 mM (red)

When evaluating the same system in 2,500 cells/well (Figure 4) it can be observed that the CI of treated neoplastic melanocytes decreases only to 85%.

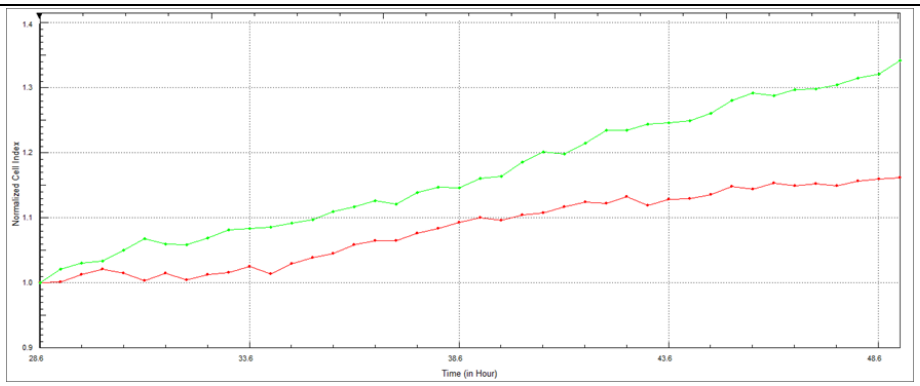


**Figure 4.**
Primary melanocytes isolated from melanoma tumour, seeded at 2,500 cells/well (green) in the presence of dacarbazine 0.25 mM (red)

When going further in our evaluation we can observe in the 5,000 cells/well system (Figure 5) that there is almost no effect for the tested drug in terms of cellular proliferation. Moreover, in the 10,000 cells/well system where one can observe even a slight proliferation in presence of the cytostatic (Figure 6).

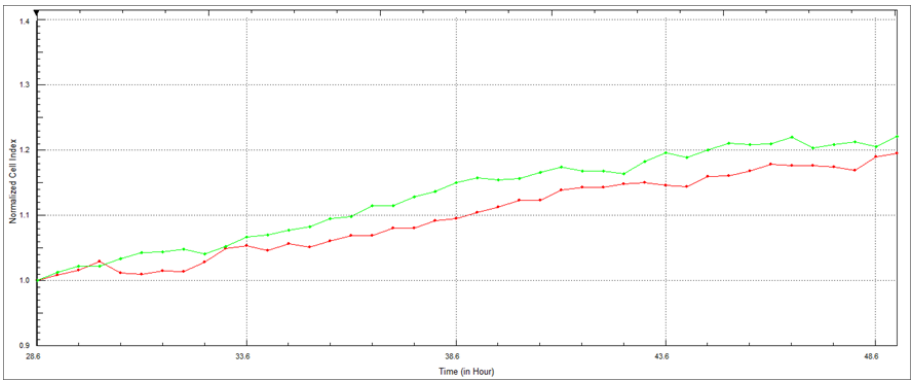


**Figure 5.**
Primary melanocytes isolated from melanoma tumour, seeded at 5,000 cells/well (green) in the presence of dacarbazine 0.25 mM (red)

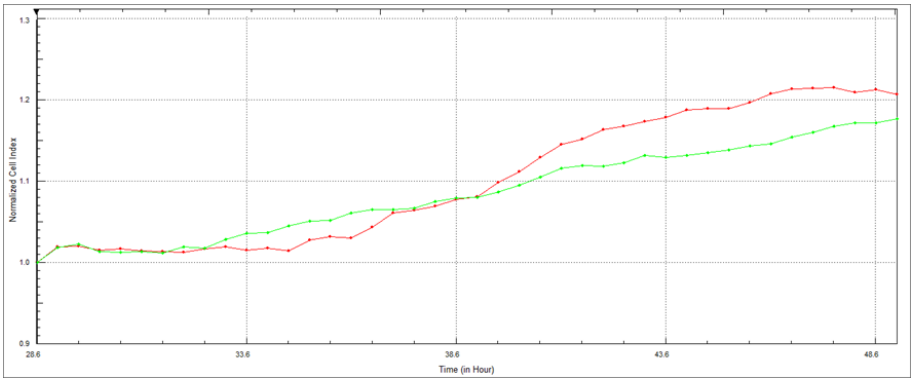


**Figure 6.**
Primary melanocytes isolated from melanoma tumour, seeded at 10,000 cells/well (green) in the presence of dacarbazine 0.25 mM (red)

## Conclusions

There are few literature data regarding the impedance measurement of melanocytes cellular behaviour. There are few studies that have used this new technology for investigating drug effects in epithelial and melanoma cancer cells [19] or other experimental therapies [20]. We present herein the impedance measurement as a potential assay for studying the sensitivity of melanocytes isolated from freshly excised tumours to standard drugs. Cellular impedance method is a reliable and rapid

diagnostic method for the monitoring of dynamic cell behaviour following drug exposure.

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