Selected Biosensors for Neurotoxicity Testing

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Abstract. *In vitro neurotoxicity testing and toxicity effect quantification plays an important role in many disciplines of biomedicine as an alternative to in vivo methods. The principle of the majority of in vitro methods corresponds to the basic concept of biosensors. i.e. measured quantity is by means of biological sensing element transformed to physical quantity easily measurable by electrical methods of measurements. Two types of biosensors suitable for neurotoxicity measurements are described in the paper. A common feature for both types is an application of the living cell as biological sensing element. In first type of biosensor the morphology of cell is evaluated using image processing methods known as videometry. In the second type of biosensors the electrical impedance of cells using an improved version of an ECIS (Electric Cell-substrate Impedance Sensing) method is a measure of toxicity effects. The results of experiments with biosensors using videometry and proposal for improvements of ECIS based biosensors are included in the paper.*

Keywords: In vitro neurotoxocity testing, Biosensors, Impedance of cells, ECIS method

1. Introduction

Neurotoxicity is defined as any adverse effect on the chemistry, structure and function of the nervous system during development or at the maturity induced by chemical or physical influences. For both economic and humane considerations there has been growing interest in alternatives to the use of animals in toxicity testing of chemical agents. Tissue culture has the potential to replace animal testing, but for the success of *in vitro* approaches new and sensitive methods to detect cellular activities are required.

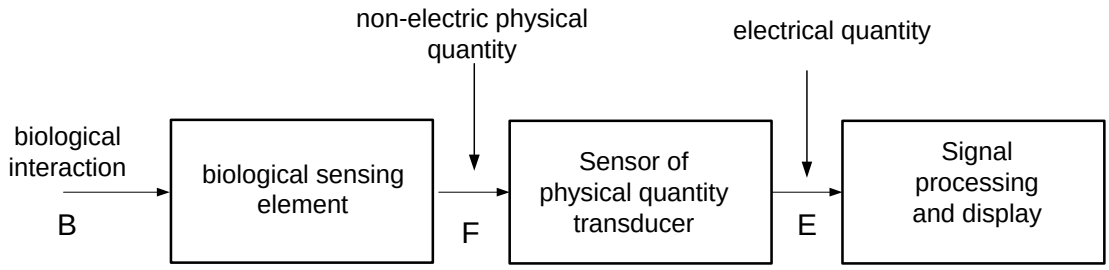

Fig.1 Block diagram of the typical biosensor

For the detection of cellular activities the concept of biosensors can be used. As it is well known *biosensors* (Fig.1) are devices incorporating a biological sensing element coupled to a variety of transducers (sensors) which convert a biological interaction into an easily measurable electrical signal. For the neurotoxity testing by *videometric* methods biological sensing elements are spinal ganglions. Spinal ganglions are clusters of nerve cells – neurons with nerve fibres sprouts called neuritis (Fig. 2). The change of neuritis morphology is very sensitive indicator of toxicity of the tested substance.

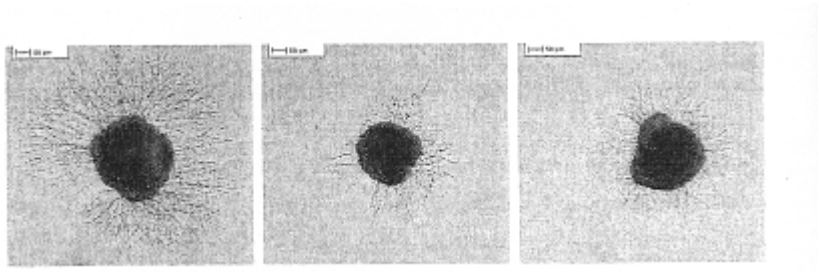

Fig. 2 The morphological changes of ganglion caused by toxic substances. (On the left - reference ganglion, in the middle - ganglion damaged by the effect of *TPPS*$_4$, on the right - the effect of *Photosan*).

Typical biological sensing elements for testing toxicity by *impedance methods* (ECIS) are fibroblastic V79 cells. The measure of toxicity is time response function of impedance when cells are exposed to toxicants.

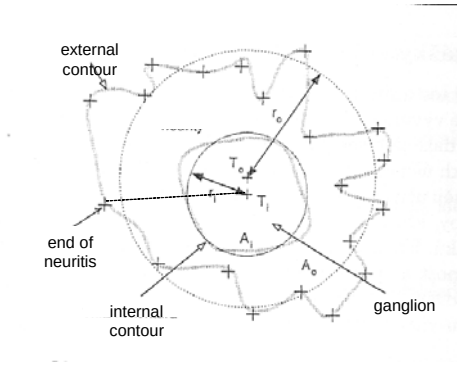

Fig. 3 Internal and external contours

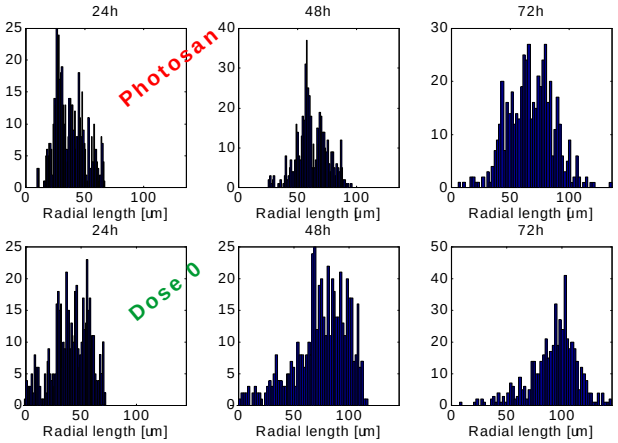

Fig. 4 Histograms of radial length of neuritis. Dose 0- reference, upper row- ganglions affected by Photosan

2. Biosensors based on image processing (videometry)

The degree of toxicity is estimated from morphological changes of neuritis exposed to toxic substance. The goal is to find quantitative parameters of ganglion geometry satisfactorily expressing the degree of toxicity of tested substance. The changes of the radial length of neuritis (Fig. 4) can be used as a measure of toxicity [1]. Another possibility is an analysis of *contours.* In case of ganglion the *external* contour is an outline connecting the endpoints of neuritis and internal contour is a circle approximating ganglion shape (Fig. 3).

Methods based on analysis of contours

Describing the contour by equation in polar coordinates analysis the discrete Fourier transform (DFT) or wavelet transform (DWT) can be used. By interpolation between endpoints we obtain continuous function c(φ) in polar coordinates. Then continuous wavelet transform of function *f(t)* defined by Equ.1 can be used.

$$Wf(\tau,s) = \int_{-\infty}^{+\infty} f(t)\frac{1}{\sqrt{s}}\overline{\psi}(\frac{t-\tau}{s})dt \qquad (1)$$

where s is scale (amplitude) parameter and τ determines position of mother wavelet $\psi(t)$

WT with properly chosen mother wavelet allows description of contour by minimum number of coefficients [2].

Descriptor CWT

In this approach the contour is divided to N segments having length *k* and for each segment transformation (1) is applied. The *descriptor CWT* is then defined by relation

$$CDc(\tau,s)=\sum_{k=1}^{N}\int_{k}^{k+1}c(k)\frac{1}{\sqrt{s}}\psi(\frac{t-\tau}{s})dt \qquad (2)$$

The maximum values of coefficients are then used for the characterization of contour.

Skeleton

By plotting local maximums of absolute values of descriptors in coordinate τ for *each* particular scale *s* so called *skeleton* (Fig. 5, on the right) is created.

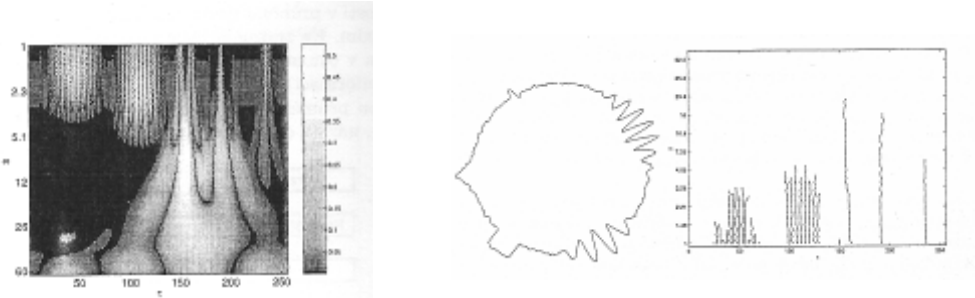

Fig. 5, left: 3-D graph of WT coefficients (wavelet Mexican hat). middle: simulation of contour radial noise. right: skeleton lines – ridges of maxima.

The advantage of skeleton description is simple elimination of noise (stochastic variation of radial length). The noise causes the appearance of skeleton line for small values of s which are then easily separated from long lines – attributes of contour [3].

3. Biosensors based on measurement of cells impedance

In this case the biological sensing element is a monolayer of cells between electrodes located in wells (Fig. 6). The presence of cells increases impedance due to isolation properties of cell membranes. Ideally, the effect of individual cell on the impedance should be observable (spatial resolution). The arrangement of electrodes using "spread resistance" principle (known from semiconductor resistivity measurement or from mercury drop polarography) can fulfill this task. Two (gold) electrodes; miniature active electrode (diameter 250 μm) and large reference one (area approx. 300 times larger) are used. The current density through active electrode is much higher thus only processes in vicinity of it affect the impedance (Fig. 6).

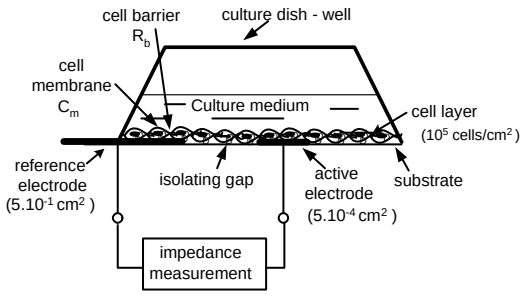

Fig.6 The principle of ECIS [4] (Patent US 7,399,631 B2)

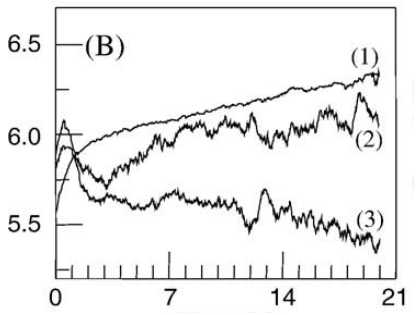

Fig.7 The change of C_m [nF] with time [h] (f = 4 kHz) (line 1-no cells, line 2,3- cell culture V79)

Impedance is measured with a weak AC signal (1 μA) in frequency range from 10 to 10^5 Hz. When cells attach and spread on and between electrodes, their insulating membranes

constrain the current, forcing it to flow beneath and between the cells that must be anchored and spread upon substratum. A limited population of cells (1 to 1,000) is measured at the time. This results in impedance changes that can be readily measured and used to quantify cell behavior.

The perspectives of ECIS method and problems to be solved

Biosensors using ECIS concept is perspective but not yet as widely used as Surface Plasmon Resonance biosensors. Further improvement of the ECIS method should solve following tasks:

1. Asymmetrical geometry of electrodes might lead to the dependence of impedance on polarity and in consequence to the presence of DC component of electrode current ("rectifier effect"). The remedy: new symmetrical arrangement of electrodes.
2. The research of feasibility of replacement expensive instrumentation by modern impedance measuring IC (e.g. impedance converter AD 5933) is in progress. At the same time the parasitic impedances of leads will be eliminated.
3. Information capacity of impedance of cell is huge and can be fully exploited by modern methods of signal processing and then widely applicable (besides toxicity testing also e.g. in cells wounding research, cell motility and spreading, etc.).
4. Finding the quantitative factors for evaluation of toxicity from the measured impedance is a difficult task requiring a lot of experimental work and the consensus of experts.

4. Results and conclusions

Biosensors for toxicity testing using morphological changes of cells are most widespread methods of in vitro toxicity testing. The transduction of cell morphology to image processing (videometry) offers more information, but requires complicated instrumentation and signal processing. The biosensors transforming cell morphology changes to impedance are much simpler and less demanding. They are just on the beginning on the era of their application in toxicology and represent the perspective orientation of in vitro toxicity testing.

But in both approaches finding of "universal" toxicity quantification parameter and method is extremely difficult, mainly due to the problems with time demanding and even dangerous experimental activities necessary for verification of the proposed toxicity criterion. At the present situation the choice of toxicity testing procedure depends to large extent on the type of toxic agent.

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