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Development of real-time monitoring of alkaline phosphatase (ALP) biomarker released from cells based on microelectrode arrays for clinical analysis

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Abstract

Alkaline phosphatase (ALP) biomarker is a membrane-bound enzyme widely distributed in the tissues of living organism. Abnormal level of ALP indicates diseases such as liver dysfunctions, bone disorders, ulcerative colitis, acute kidney injury and can diagnose cancer at early stage. There is thus a demand for a reliable technique to accelerate the sensing of ALP in point-of-care applications. Such methods although sensitive are costly process, and based on optical detections, which hard to be miniaturized. This work presented shows a method that integrates cell culture and electrochemical techniques to develop real-time detection of monitoring ALP released from cells.

Key words: Alkaline phosphatase, cell viability, microelectrode arrays, impedance, electrochemical sensor.

Introduction

The purpose of our work is to develop an electrochemical method that will pave the way to perform direct, cost effective, simple, monitoring of ALP biomarker from release in cells for point-of-care applications. Our approach is to integrate cell culture and electrochemical techniques to develop real-time detection of monitoring ALP released from living cells. The integration of these techniques can enhance the detection of biological targets based on the electrochemical change of electrode interfaces.

Our integrative approaches will take advantage of microelectrode arrays technology, facilitating the yield of recordings, signal shape, and signal-to-noise ratio. Electrochemical impedance devices, has 40 electrodes distributed in 8 wells that allow continuous visualisation of cell adhesion, spreading, proliferation, and detachment. Amperometric sensor has three-electrode system to monitor cellular signal transduction under a monolayer of cell culture conditions. The amperometric sensor application offers solutions that can be used in screening cellular ALP expression detected by electroactive species and hence avoid, radioactive, antibodies, mRNA tools that are presently used in clinics.

By optimizing impedance and amperometric parameters that investigate the cells confluence and concentrations of ALP, we hope to obtain sufficient real-time monitoring of ALP under cellular behavior in which provide a huge data for designing a portable prototype sensor for monitoring ALP from release in cells. The concentration will be compared to those available in state-of-the-art literature and shown in Table 1. It will present a linear range between the concentration of ALP and number of viable cells. Optimal results will be conducted with the patch clamp techniques. To the extent of our knowledge, few experiments is so far available on real-time monitoring ALP level in microelectrode arrays technology.

Tab. 1: Table literature reviews

Methods	Linear Range U/L	Limit of Detection U/L	Ref.
Fluorescent assay	3.0 - 27.0	0.19	[1]
Colorimetric assay	1.5–20	0.78	[2]
Chemiluminescence assay	2 – 25	2	[3]
Photoelectrochemical assay	3×10^{-4} - 1	0.0001	[4]
Electrochemical assay	1- 500	0.5	[5]

Cell Viability Optimization

For cells growth, media is mixed with supplements, which affect fluorescent signal shifts. The cells density affected by working

area as well. Chosen the optimal density of cells that can stand the microtiters 4 days are performed and shown in figure 1. Reszurin needs several hours to be reduced to resorufin.

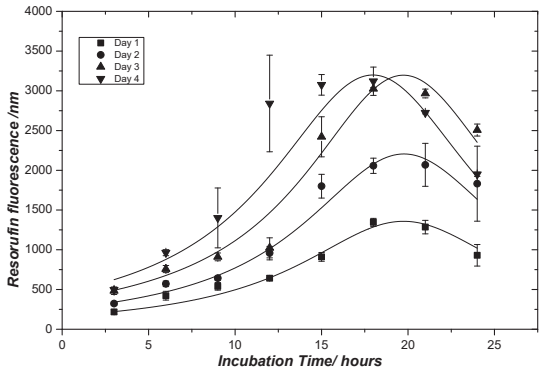

Fig. 1. Graph of resazurin reduction vs serial incubation time for 4 days at cell density of 80k cell/ml.

ALP measurements

To investigate secreted ALP enzyme levels in the living cells, calibration experiments are conducted. Figure 2. Shows the optimal concentration of ALP. Figure 3 shows the optimal concentration of substrate.

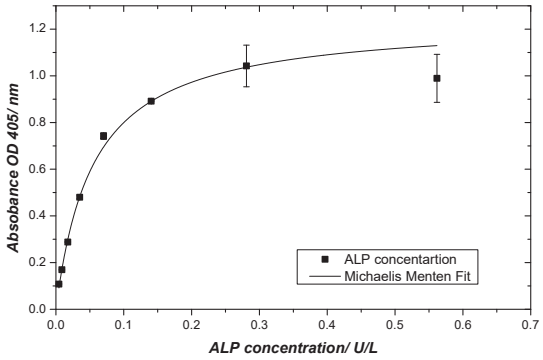

Fig. 2. Michaelis–Menten saturation of absorbance density vs serial concentration of ALP.

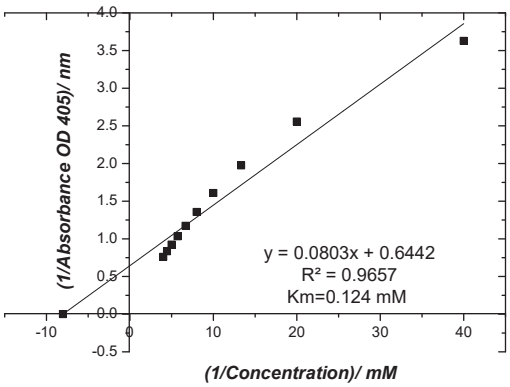

Fig. 3. Lineweaver–Burk plot of absorbance density vs serial substrate concentrations.

Cell-Substrate Impedance Sensor

impedance cell-based biosensor assays are performed in (electrde). Fibroblast cells-Balb/c3T3 at the optimal density 80K cells/ml are seeded on a microelectrode surface of 0.8 cm^2.

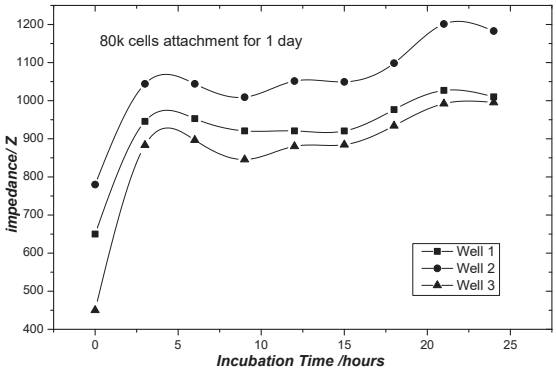

Fig. 4. Preliminary data of impedance at 8000 Hz frequency vs incubation time of 80k cell/ml and monitored for one day.

Future work

Preliminary data of microelectrode arrays need to be repeated. Quantity of secreted ALP need to be correlated with cell density. Amperometric optimization will be then conducted and compared to those of optical results. Shifts of current (nA) in amperoemtric detection will be compared to those in patch clamp techniques.

Acknowledgement

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Conclusion

In our opinion the method with the ability of detecting ALP level directly with an inexpensive detection tool will ultimately be the future of point of care applications.

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