The Development of Electrochemical Aptasensor based on DNA Aptamers modified by Redox Markers for Detection of Leukemia Jurkat Cells.

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INTRODUCTION

LEUKEMIA

Leukemia is one of the most common and aggressive oncological disease. Acute lymphoblastic leukemia (ALL) is an aggressive form of leukemia that originates in a single B- or T-lymphocyte progenitor.

JURKAT cells

Jurkat cells are an immortalized line of T-lymphocytes usually used for studying acute T-cell leukemia, T-cell signaling or expression of chemokine receptors. The cell line was established in 1970s from peripheral blood of 14-year-old boy with T-cell leukemia.

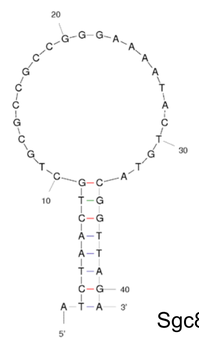
Protein tyrosine kinase 7 (PTK7)

Extracellular domain | TM domain | TK domain

PTK7 is a potential surface diagnostic biomarker with predictive value and a promising drug target in cancer research. PTK7 is highly expressed in cancerous tissues (colon cancer, melanoma, breast cancer, acute myeloid leukemia, acute lymphoid leukemia).

APTAMERS

Aptamers are single stranded DNA or RNA molecules that are obtained *in vitro* with an evolution process SELEX (Systematic Evolution of Ligands by EXponential enrichment).



APTAMER sgc8c

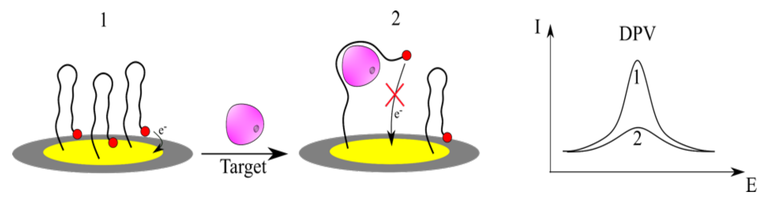
The sgc8c DNA aptamer for PTK7 was developed by Shangguan (2007) and can be used instead of antibody as a receptor in biosensor for recognition of JURKAT leukemic cells.

Sgc8c (Eurogentec, Belgium)

LABELED APTASENSOR

Sgc8c aptamers are modified by two important types of the redox markers:

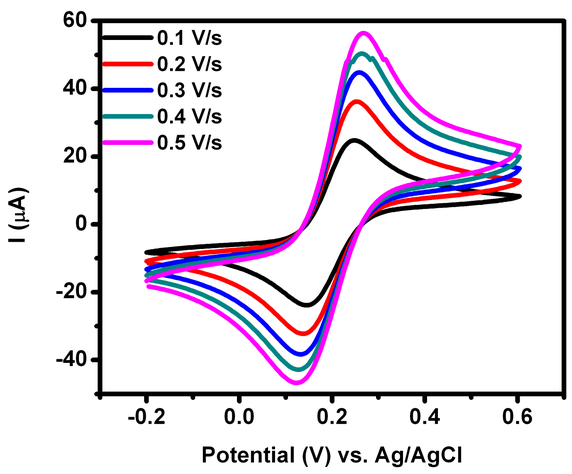
- By methylene blue: SH-sgc8c-MB
- By ferrocene carboxylic acid: biotin-sgc8c-NH_2



The scheme of electrochemical aptasensor. **Signal off:** binding reduces the current from the redox tag (red circle). Aptasensor before binding of target (part 1) and after binding the cells (purple object, part 2). DPV current changes correspond to binding phenomenon.

RESULTS

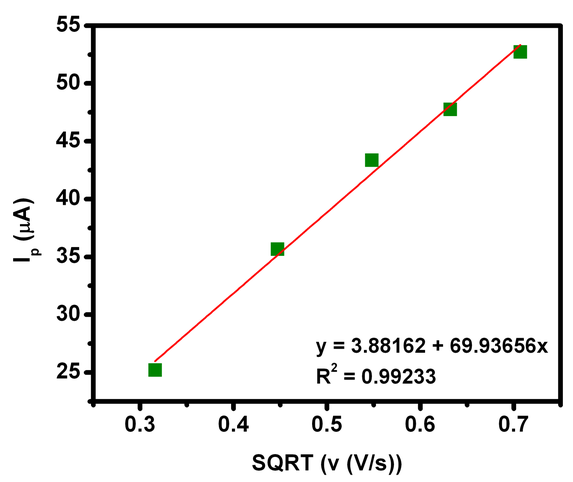
THE ELECTROCHEMICAL PROPERTIES OF SENSING LAYER



The voltammograms of bare gold electrode recorded in 5 mM (1:1) $[Fe(CN)_6]^{3-/4-}$ as a redox probe in 0.05 M KCl solution at various scan rates.

Electrochemical active area, A_{eff}, was 0.02 ± 0.001 cm².
The actual geometrical area of the gold electrode is 0.031 cm².

The ratio of active to geometrical surface area obtained by electrochemical measurement: **0.61 ± 0.06** (Krejci et al., 2014).



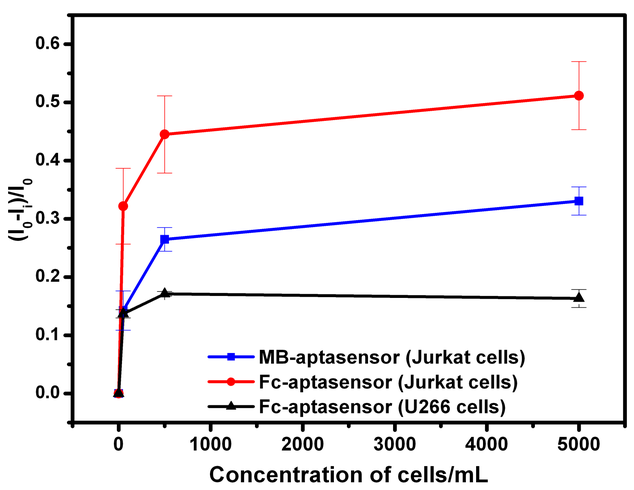
Dependence of the peak current versus square root of scan rate. The linear fit (red line) of the data and the fitted parameters are given in the figure.

DISCUSSION and CONCLUSION

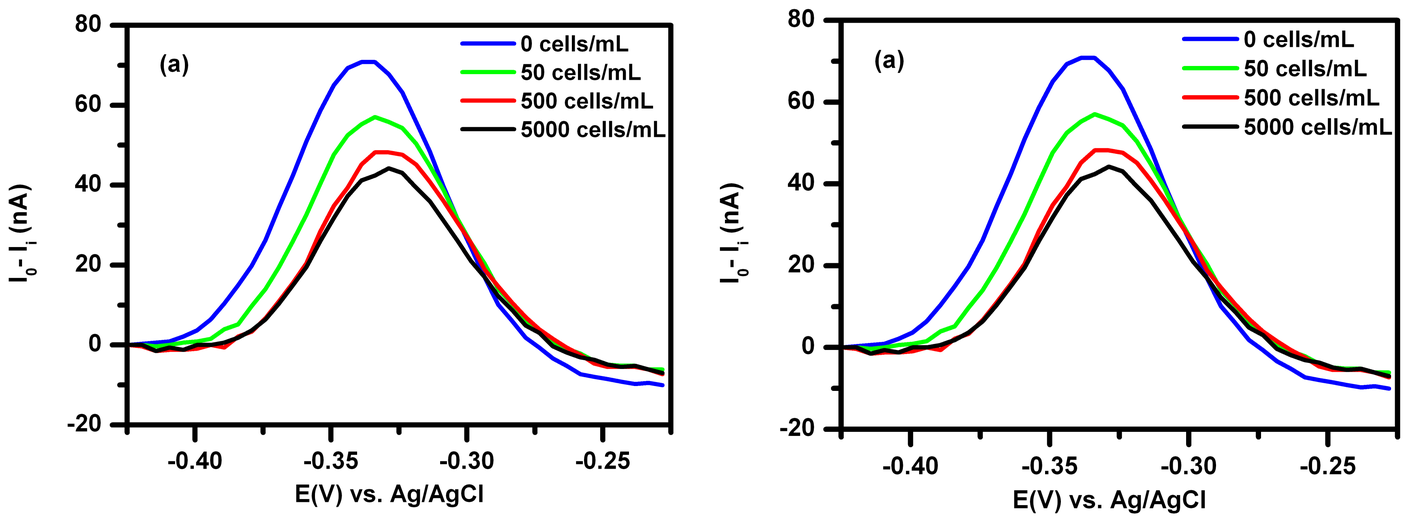
DETECTION of Jurkat cells

We used differential pulse voltammetry (DPV) as detection method.

1) Baseline: DPV signal obtained immediately after the formation of sensing layer in blank working buffer.
2) The interaction of PTK7 receptor on the membrane of leukemic cells with the aptasensor .
3) After one hour of incubation of the aptasensor with the cell suspension the electrochemical properties were efficiently detected by DPV (graph (a) and (b)).
4) **In our research, we tested Jurkat (T-cell, acute lymphoblastic leukemia) and the control U266 (B-lymphocytes, a PTK7 negative cell line) cell lines.**



The relative changes of the peak current intensity of the two types electrochemical aptasensors vs. cell concentration. The error bars were obtained from three experiments. I_0 is peak current prior addition and I_i after addition of the cells in a respective concentration.



Differential pulse voltammograms of methylene blue (a) and ferrocene carboxylic acid (b) modified aptamer at different Jurkat cell concentrations in working buffer at pH 7.4. DPV has been baseline corrected.

LIMIT OF DETECTION (3.3 S/N)

(MB-aptasensor) 38±8 cells/mL **(Fc-aptasensor) 37±6 cells/mL**

The redox-active units are responsible for electron production and signal amplification in an electrochemical biosensor. The signal changes are influenced by the binding properties of the Jurkat leukemic and U266 control cells to the aptamer binding site.

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2. Bábelová, L.; Sohová, M.E.; Poturnayová, A.; Buríková, M.; Bizík, J.; Hianik, T. Label-free electrochemical aptasensor for Jurkat cells detection as a potential diagnostic tool for leukamia. *Electroanalysis* **2018**, *30*, 1-10.