IMPEDIMETRIC LECTIN-BASED BIOSENSORS FOR CANCER-ASSOCIATED *O*-GLYCANS

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Abstract

During oncogenesis, several changes occur within cells. Specifically in protein glycosylation, differential expression of enzymes catalyzing the addition of sugars to the protein result in the synthesis of aberrant glycan structures. When the ensuing glycoproteins are secreted into the blood stream, these abnormal glycostructures can act as valuable cancer biomarkers. Among the best known pan-carcinoma glycobiomarkers are truncated *O*-glycans, namely STn, Tn and T antigens.

Detection of aberrant *O*-glycoproteins in serum can be successfully performed by using lectin biosensors, as lectins show high selectivity towards particular glycan structures. This work gathers and presents three lectin-based impedimetric biosensors for the selective detection of STn, Tn and T antigens, using *Sambucus nigra* agglutinin (SNA), *Vicia villosa* agglutinin (VVA) and *Arachis hypogeae* agglutinin (PNA) as biorecognition elements, with specificity for STn, Tn and T antigens, respectively [1-3]. The binding event between each lectin and the corresponding aberrant *O*-glycan was monitored by electrochemical impedance spectroscopy, measuring the increase in the biosensor's impedance after incubating the samples. The increase in impedance was related to the lectin-glycan complex formation. The developed biosensors showed high selectivity, high discrimination capacity between controls and cases, fast assay time, label-free detection and high flexibility in the construction procedure. By using all biosensors for the analysis of the same cancer type, different glycosylation patterns could be observed, according to lectin specificity.

Biosensor construction

cancer-associated truncated *O*-glycan	lectin immobilized on the biosensor	model glycoprotein used to monitor complex formation
STn	SNA	bovine submaxillary mucin; human transferrin
Tn	VVA	asialofetuin; asialo-bovine submaxillary mucin
T	PNA	asialofetuin

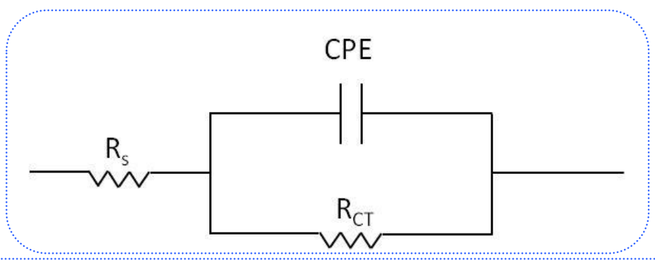


Figure 1 – *Randles* equivalent circuit for the developed biosensors. R_s – resistance of the electrolyte solution; CPE – constant phase element; R_{CT} – charge transfer resistance.

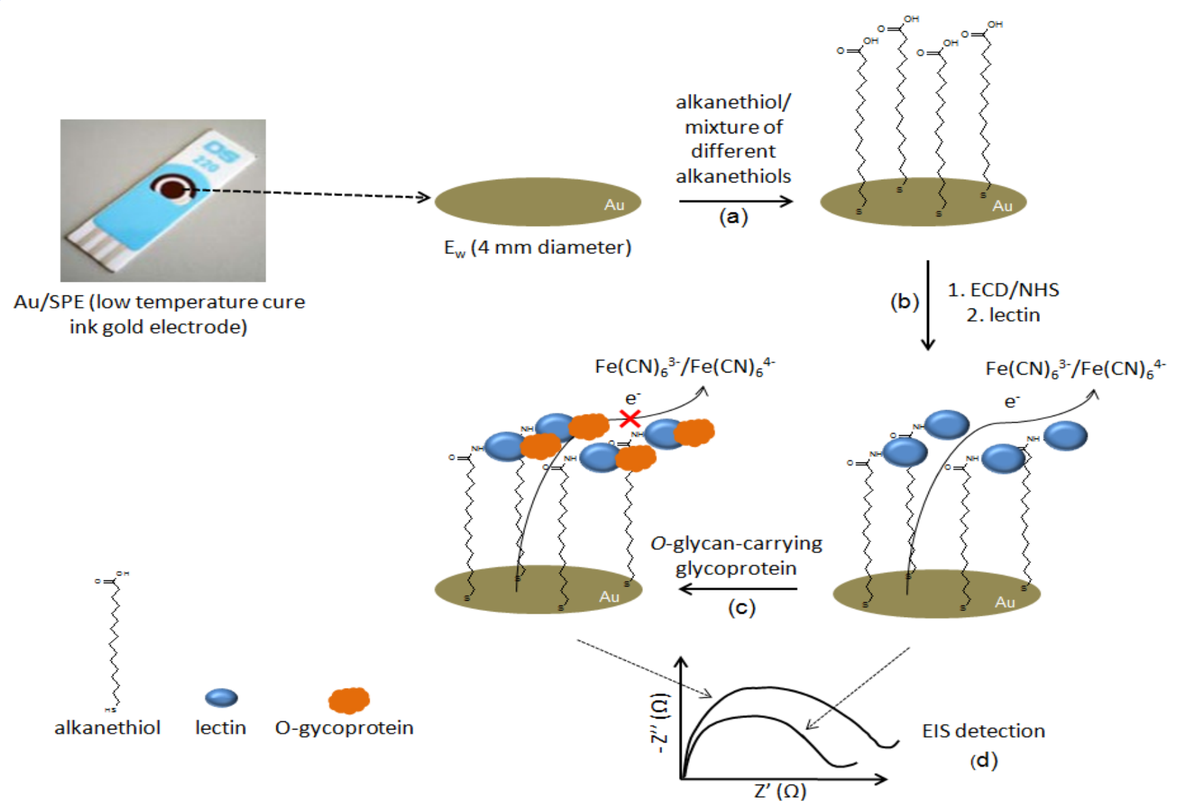


Figure 2 – Schematic diagram describing the construction of each lectin biosensor and detection of aberrant *O*-glycans by EIS: (a) alkanethiol/mixed alkanethiols self-assembled monolayer is formed via incubation of screen-printed electrodes for 24 h; (b) the carboxylic acid end of the alkanethiols are activated with ECD and NHS to allow covalent binding with the lectin; (c) the truncated *O*-glycan present in glycoproteins is captured based on the affinity of the lectin to the referred structure; (d) the formation of the complex lectin-truncated *O*-glycan is monitored by the increase in the electrode impedance (by electrochemical impedance spectroscopy).

Results

SNA biosensor | **VVA biosensor** | **PNA biosensor**

selectivity

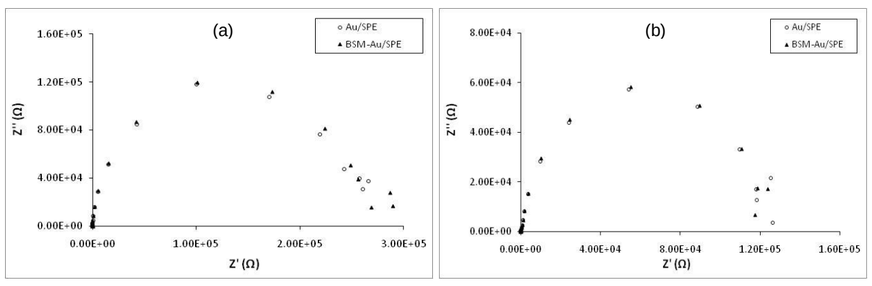
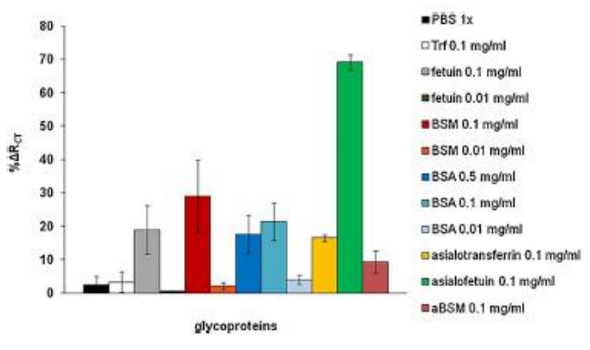
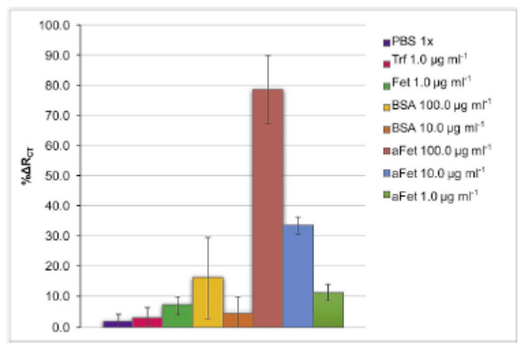


Figure 3 – Nyquist plots obtained before and after incubating the blank biosensor (with no lectin) with BSM solutions (a) 0.01 μg ml^{-1} and (b) 1.0 μg ml^{-1}, for 5 min at room temperature.

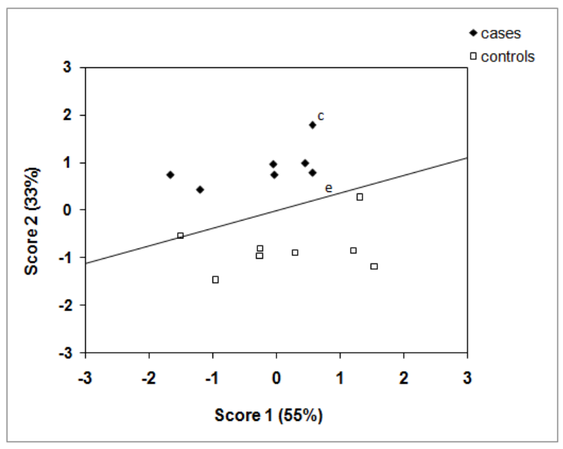




Figures 4 and 5 – Response for several glycoprotein solutions, incubated for 10 min. Error bars indicate standard deviations of duplicate measurements with two independent biosensors for each solution.

sample analysis

Figure 6 – Graphical representation of the first two scores of a PCA performed on the impedimetric data from sample analysis using the SNA biosensor. Each point represents an individual analysis of a sample; (a) – breast carcinoma, (b) – retroperitoneal located malignant tumour, (c and e) – pools with 25 different cancer samples, (d) – cervical-uterine carcinoma.



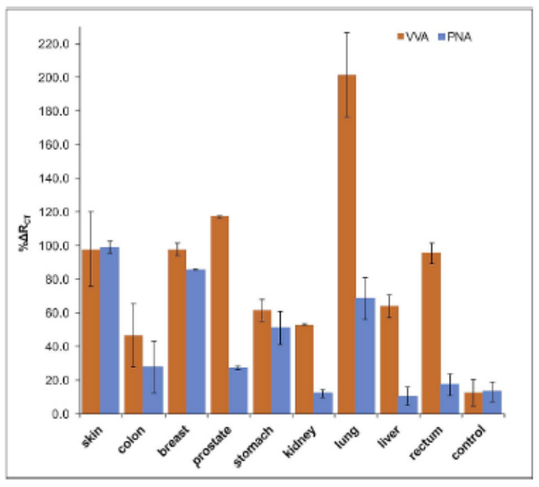


Figure 7 – Results obtained in sample analysis for VVA and PNA biosensors. Each sample pool refers to a type of carcinoma. Ctrl represents a pool of samples from healthy donors. Error bars indicate standard deviations of duplicate measurements with two independent biosensors for each solution.

References: [1] M. Luísa S. Silva, Evelin Gutiérrez, José A. Rodríguez, Catarina Gomes, Leonor David. Biosens. Bioelectron. 57 (2014) 254-261. [2] M. Luísa S. Silva, María G. H. Rangel. Sens. Actuators B 252 (2017) 777-784. [3] María. G. H. Rangel, M. Luísa S. Silva. Biosens. Bioelectron. 141 (2019) 111401.