Detection of cancer-associated glycobiomarkers using lectin-based biosensors

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• Introduction

Lectin biosensors are attractive devices for the detection of cancer-associated glycobiomarkers in serum since they combine the advantageous aspects of biosensors (portability, easy use in point-of-care analysis, low sample requirement) with the high selectivity of lectin biorecognition. This work presents three lectin-based impedimetric biosensors for the selective detection of specific aberrant cancer-associated *O*-glycans, namely STn, Tn and T antigens, which are well-established pan-carcinoma biomarkers. For these three biosensors, *Sambucus nigra* agglutinin (SNA), *Vicia villosa* agglutinin (VVA) and *Arachis hypogeae* agglutinin (PNA) were used as biorecognition elements, with specificity for STn, Tn and T antigens, respectively. 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 [1-3].

• 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

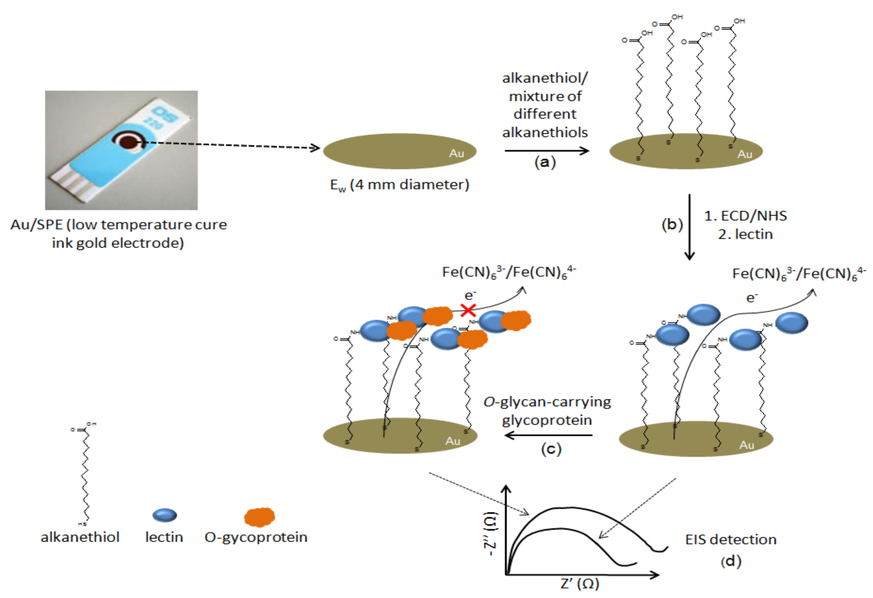


Figure 1 – Schematic diagram describing the construction of each lectin biosensor and detection of aberrant *O*-glycans by EIS: (a) alkanethiol/mixed alkanethiols self-assembled monolayer is formed via incubation of screen-printed electrodes for 24 h; (b) the carboxylic acid end of the alkanethiols are 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).

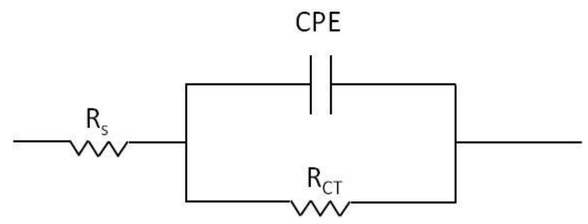
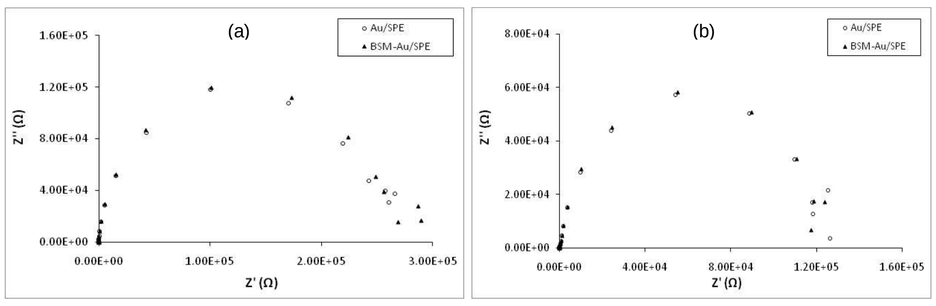


Figure 2 – *Randles* equivalent circuit for the developed biosensors. R_s – resistance of the electrolyte solution; CPE – constant phase element; R_{CT} – charge transfer resistance.

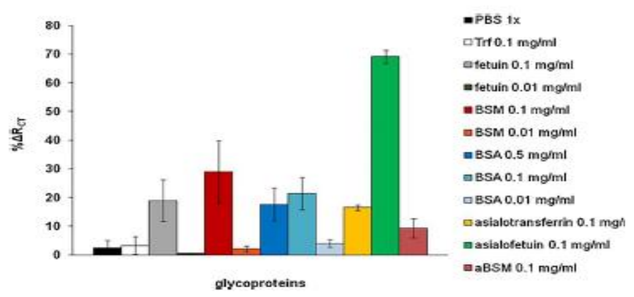
• Selectivity

SNA biosensor

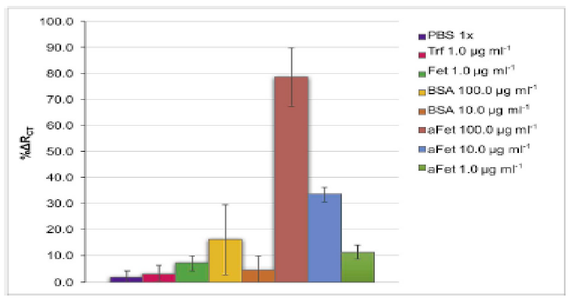


Figures 3 and 4 – Nyquist plots obtained before and after incubating the blank biosensor (with no lectin) with BSM solutions (a) 0.01 μg ml^{-1} and (b) 1.0 μg ml^{-1}, for 5 min at room temperature.

VVA biosensor



PNA biosensor



Figures 5 and 6 – 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

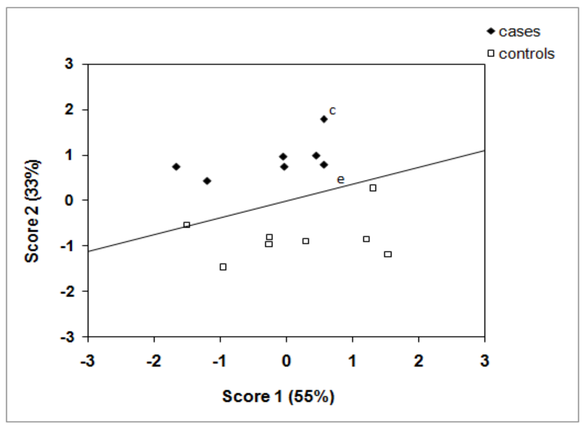


Figure 7 – Graphical representation of the first two scores of a PCA performed on the impedimetric data from sample analysis using the SNA biosensor. Each point represents an individual analysis of a sample; (a) – breast carcinoma, (b) – retroperitoneal located malignant tumour, (c and e) – pools with 25 different cancer samples, (d) – cervical-uterine carcinoma.

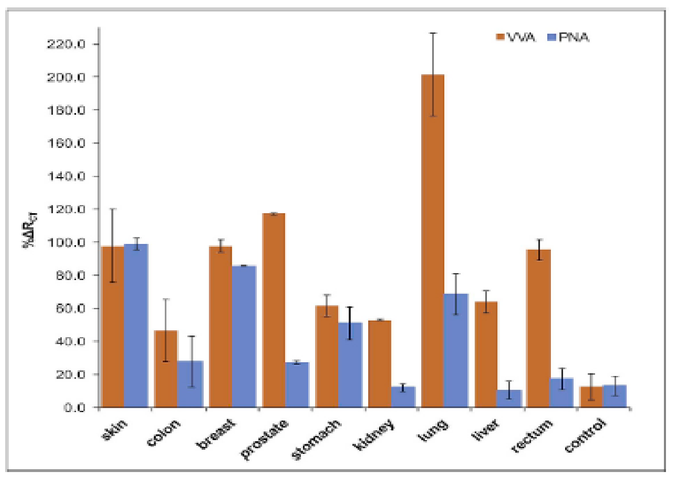


Figure 8 – Results obtained in sample analysis for VVA and PNA biosensors. Each sample pool refers to a type of carcinoma. Ctrl represents a pool of samples from healthy donors. Error bars indicate standard deviations of duplicate measurements with two independent biosensors for each solution.

• Conclusions

1. All biosensors were constructed following the same general procedure, demonstrating its high versatility.
2. The three biosensors correctly discriminated samples from healthy donors and from cancer patients with different carcinomas, showing high selectivity towards the antigens STn, Tn and T.
3. Using the three lectin biosensors in the analysis of the same sample allowed to characterize the glycosylation profiles of glycoproteins in the diverse types of carcinomas.

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.