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 hypogaea 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

<table>
<thead>
<tr>
<th>cancer-associated aberrant O-glycan</th>
<th>lectin immobilized on the biosensor</th>
<th>model glycopolymers used to monitor complex formation</th>
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<tbody>
<tr>
<td>STn</td>
<td>DNA</td>
<td>bovine submaxillary mucin; human transferrin</td>
</tr>
<tr>
<td>Tn</td>
<td>VVA</td>
<td>asialofetuin; bovine submaxillary mucin</td>
</tr>
<tr>
<td>T</td>
<td>PNA</td>
<td>asialofetuin</td>
</tr>
</tbody>
</table>

Figure 1 – Schematic diagram describing the construction of each lectin biosensor and detection of aberrant O-glycans by EIS: (a) alkanethiolated alkane thiol self-assembled monolayer is formed via incubation of screen-printed electrodes for 24 h; (b) the carboxylic acid end of the alkanethiols is activated with EDC and NHS to allow covalent binding with the lectin; (c) the truncated O-glycan present in glycopolymers 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).

• Selectivity

SNA biosensor

VVA biosensor

PNA biosensor

Figure 2 – Schematic equivalent circuit for the developed biosensors. $R_s$ – resistance of the electrolyte solution; $CPE$ – constant phase element; $R_w$ – charge transfer resistance.

Figure 3 and 4 – Nyquist plots obtained before and after incubating the blank biosensor (with no lectin) with BSA solutions (a) 0.01 µg mL⁻¹ and (b) 1.0 µg mL⁻¹, for 5 min at room temperature.

Figure 5 and 6 – Response for several glycopolymer 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) – non-representative located malignant tumour, (c) and (d) – pools with 25 different cancer samples, (e) – cervical-uterine carcinoma.

• 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 the glycoproteins in the diverse types of carcinomas.