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Application of ultrasensitive electrochemical biosensors for specific analysis of a glycan on a prostate-specific antigen

Prostate cancer (PCa) is the second most common cancer worldwide, accounting for more than 1.1 million cases in 2012. The statistics says, that about 1 man in 7 will be diagnosed with PCa during his lifetime with the preferential development in older men. The symptoms of early PCa are very mild of even absent and majority of PCa cases (92%) are found when the disease is confined to the prostate and nearby organs. Nowadays, the gold standard for PCa diagnostics is serological measurement of prostate specific antigen (PSA). However, PSA serum level varies to a high extent with age and cannot provide a clear difference between malignant and benign (benign prostate hypertrophy) cases. Hence, the improved efforts for development of novel diagnostic tools with higher specificity and sensitivity is evident.

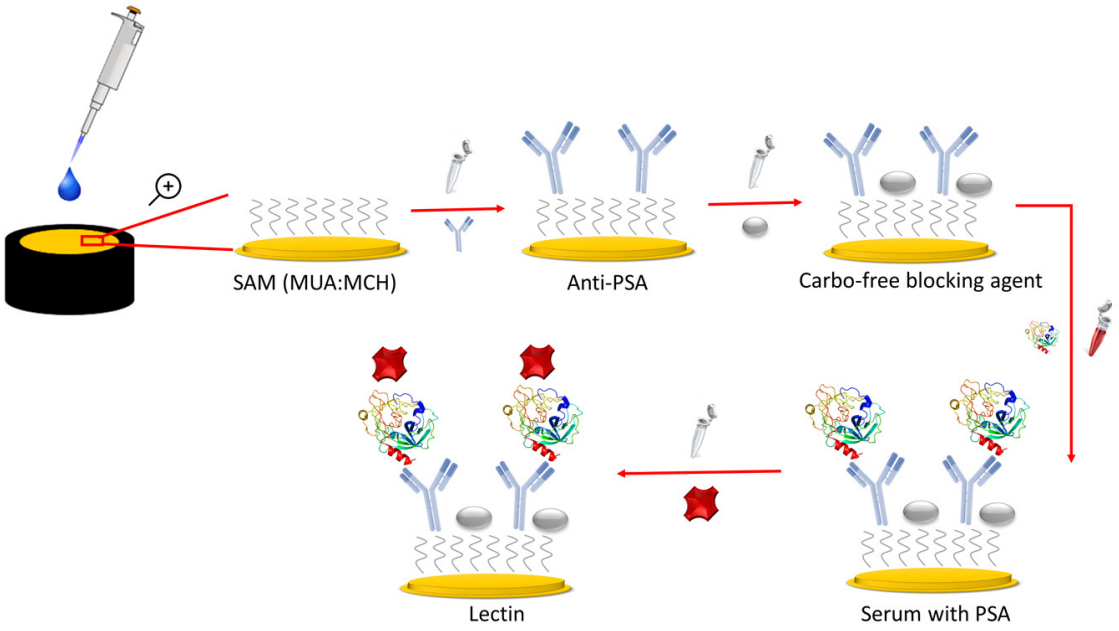

Fig. 1. Schematic illustration of lectin-based immunosensor preparation.

Cancer cells frequently show altered and dysregulated glycosylation of their cell surface and this phenomena belongs to universal attribute of pathological processes and malignant transformation in a human body. The determination of specific tumour-associated glycoforms of proteins secreted by cancer cells may either improve the specificity of known cancer biomarkers (e.g. PSA) or allow the discovery of new cancer glyco-biomarkers.

Herein we present lectin-based electrochemical immunosensor strategy with PSA glycoprofiling in serum samples (Fig. 1). Our approach is based on electrochemical impedance spectroscopy enabling the detection of PSA down to 100 ag/mL with a linear concentration range from 100 ag/mL to 1 µg/mL. Furthermore, application of lectins as specific glycan-binding proteins allow to identify a specific sub-glycoproteome and

even the type of a glycosidic linkage between terminal carbohydrates within a glycan (differentiation between α-2,6- and α-2,3-linked sialic acid). The fabricated lectin-based biosensor was applied to distinguish serum samples of PCa patients and healthy individuals. The data showed that *Maackia amurensis* agglutinin (MAA) recognizing α-2,3-terminal sialic acid can be utilized to differentiate between these two sets of samples since the MAA/PSA response obtained from the analysis of the PCa samples was significantly higher (5.3-fold) compared to the MAA/PSA response obtained by the analysis of samples from healthy individuals.

Besides, the comparison of four blocking agents was performed since the reduction of non-specific interaction is crucial step of biosensor preparation. In order to create an appropriate platform we compared blocking effectivity of 1 M ethanolamine; 0.8% gelatine+0.05% Tween 20; 0.1% gelatine and a carbo-free blocking solution by monitoring a non-specific binding of SNA lectin towards the biosensor surface with an immobilized antibody. The results revealed that a commercial carbo-free blocking solution was the best one, reducing non-specific interactions 55-fold when compared to the immunosensor surface without any blocking agent applied.

To conclude, our lectin-based impedimetric immunoassay demonstrated the direct analysis of cancer-associated glycosylation status of PSA in human serum samples in a sensitive and patient-friendly way. The results showed a great potential of discrimination between PCa patients and healthy individuals based on increased level of α-2,3-terminal sialic acid present on PSA from PCa patients compared to healthy individuals with possible future application for PCa diagnostics.

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