Paper Based Magnetic Nanoparticles Biosensor for rapid Prostate Specific Antigen detection

GHADEER A.R.Y. SUAIFAN, Department of Pharmaceutical Sciences, Faculty of Pharmacy, The University of Jordan, Amman11942 JORDAN
MOHAMMED ZOUROB, Department of Chemistry, Alfaisal University, Al Zahrawi Street, Al Maather, AlTakhassusi Rd, Riyadh 11533, SAUDI ARABIA
gh.suaifan@ju.edu.jo http://eacademic.ju.edu.jo/gh.suaifan/default.aspx

Abstract Paper-based magnetic nanoparticle biosensor for the evaluation of active PSA level was designed. This biosensing configuration is based on the detection of PSA amylodic activity using a specific peptide substrate, sandwiched between magnetic nanonanoparticles and gold sensing platform placed on the top of a paper support. Upon PSA addition, an external magnet fixed at the back of the sensor support would accelerate the cleavage of the magnetic nanoparticles-peptide moieties away from the surface. Colorimetric change from black to golden color was visualized by the naked eye. This biosensor was amenable for a qualitative and quantitative PSA detection in buffer and in spiked urine samples with a lower detection limit of 1 ng/mL and 5 ng/mL, respectively. In conclusion, the designed colorimetric biosensor pinpointed superiority in criteria's including sensitivity; simplicity and fast processing time.

Key-Words: - Protease; Prostate Cancer; Prostate Specific Antigen; Colorimetric; Biosenser; Magnetic nano-particles.

1 Introduction

Proteases comprise 2% of the human genome and control a diverse array of biological processes.[1] The critical role of proteases in diseases development has been investigated and prompted their current use as a disease diagnostic biomarkers.[2-7] For example, prostae specific antigen (PSA) serine protease was proved to be a fruitful prostate cancer diagnostic biomarker. [8-13]

In male, prostate carcinoma is the leading cause of cancer-related deaths, aside from lung cancer.[14]

Normally, PSA level in healthy men blood is below $4X10^3$ pg ml. However, when level exceeds 10^4 pg ml, higher probability of prostate cancer is indicated. PSA in body fluids is presented in two forms, proteolytically-active and proteolytically-inactive[8-13]

Conventional method used to eradicate prostate carcinoma include radical prostatectomy. Yet, recurrence occurred in approximately 40% of patients after fifteen years.[15] In recurrent prostate carcinoma, an increase in proteolytically-active PSA form occurs nearly universally.[16] At present, researchers proved that sensitive detection methods with Lower Limit of Detection (LOD) <100 pg ml^{-1} could identify prostate cancer replase one year earlier in most patients with recurrence and so improving the survival rate.

Today, PSA detection is based on the use of commercial immunoassays and other approaches[17,18] Generally, these methods offer good sensitivity but do not discriminate between proteotically-active and inactive PSA forms.

Thus, a call for a simple and highly sensitive method for the evaluation of active PSA level as a tool for detecting biochemical relapse after radical prostatectomy is a challenging issue.

In this context, the development of nanomaterial based biosensor as a portable real time PSA diagnostic device would have a fruitful outcome. Thus, in this work, we developed a novel, facile and rapid paper-based PSA biosensor based on the use magnetic nanoparticles (MNPs)-peptide probe for the detection of active PSA form.

2 Materials

2.1 Experimental

Carboxyl-terminated beads (50 nm in diameter), N-hydroxysuccinimide (NHS), 1-(3-dimethylaminopropyl)-3-ethyl-carbodiimide (EDC) and plastic pH indicator strips were purchased from Sigma Aldrich (Dorset, UK). Self-adhesive magnet sheets were purchased from Polarity Magnets Company (UK). The peptide for S. Chartarum, GSGSGSGSEHSSKLQLAKGSGSGSGSC [12], was synthesized by Pepmic Co., Ltd (Suzhou, China). The self-adhesive tape was purchased from Whatman (London, U.K) and coated with a thin layer (30 nm) of gold at School of Engineering at Cranfield University. Malt extract agar and Malt extract broth were purchased from Oxoid (Amman, Jordan). Sterile filters (0.22 µm) were purchased from Millipore (Amman, Jordan). The wash/storage buffer (10 mM Tris base, 0.15 M sodium chloride, 0.1% (w/v) bovine serum albumin, 1 mM ethylenediaminetetraacetic acid, 0.1% sodium azide, pH 7.5) and the coupling buffer (10 mM potassium phosphate, 0.15 M sodium chloride, pH 5.5) were prepared from chemicals of analytical grade.

2.2 Labeling of MNPs with PSA Peptide Substrate

The MNPs suspension (1 mL) was mixed with peptide (1.0 mg / mL), the coupling agent EDC (0.57 mg / mL) and NHS (12 µg / mL). The mixture was quivered by a rollar shaker at room temperature 18 hours. Afterwhich, MNPs were washed three times with a wash buffer to remove uncoupled peptides. Finally, the MNPs were stored at 4^{o}C in a storage buffer for later application.

2.3. Fabrication of Biosensor Sensing Platform

Self-adhesive tape was coated with a golden thin layer sputtered using RF magnetron sputtering (Nordiko Ltd). Conditions 5mT pressure Argon gas. Power 100W, 5mins at Cranfield University-United Kingdom. Following this, a rectangular piece (~1.5-2 X 3 mm) was cut and stacked over a plastic physical support to fabricate the sensing platform (Fig.1B). Then, labeled MNPs-peptide solution was mounted over the gold sensing platform and left at room temperature till dryness to fabricate the sensing layer (Fig. 1C). Afterwhich, an external magnet was passed over the sensing layer from a distance of 1 cm to remove any unattached labeled MNPs-peptide moieties (Fig. 1D). After that, a round paper puncher size magnet was glued at the strip rerward underneath the gold sensing platform at a distance of 2-3 mm.

2.4. Biosensing of PSA

Different concentrations of PSA (100 pg / mL, 1ng / mL, 10 ng / mL, 100ng / mL, 1µg / mL and 5 µg /mL)) were used. After which the functionalized PSA sensor plaform will be examined.

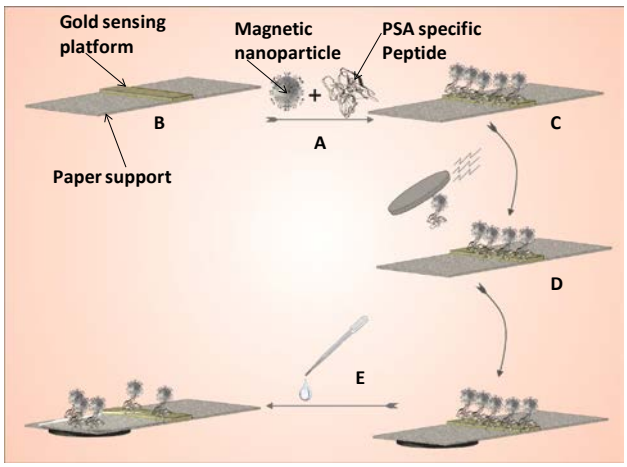

Fig.1. Mechanism of proteolytically active PSA detection using MNPs-peptide probe (**A)** Functionalisation of MNPs with PSA specific peptide substrate (**B)** Gold sensing platform. (**C)** Immobilization of functionalized MNPs on gold sensing platform placed over a paper support (**D)** Sensor platform under the effect of an external magnet to remove any unattached MNPs. (**E)**. Biosensing process.

3. Results and discussion

The designed biosensor was based on the detection of PSA amidolytic activity using MNPs-peptide probe placed on top of paper support as shown in Fig. 1. The biosensing probe was made up of PSA specific peptide substrate covalently coupled at the N-terminus with COOH-terminated MNPs and to a gold sensing platform via S-S linkage at the peptide C-terminal. This probe was placed over a paper support to enable on-site detection. A magnet

was passed over the immobilized gold sensing platform to remove any unattached MNPs. Following PSA introduction, the linkage between the MNPs and the sensor gold surface will be abolished. An external magnetic field stacked at the sensing support back will collect the cleaved MNPs-peptide moieties away from the gold sensing platform with an optical color change (Black to golden).

Application of different PSA buffer concentrations (100 pg mL^{-1}, 1 ng mL^{-1}, 10 ng mL^{-1}, 100 ng mL^{-1}, 1 µg mL^{-1}, 5 µg mL^{-1}) over the black-colored sensing platform showed an optical increase in the sensing platform golden color (Fig. 2A). This is due to the proteolytic activity of PSA, which results in the dissociation of the MNPs-peptide moieties. Detection limit determination was based on the visual evaluation of the lowest PSA concentration incapable of cleaving peptide-MNPs moieties covalently attached to the golden sensing plaform, i.e. the sensor golden surface area is invisible to the naked eye due to intact layer. Accordingly, LOD was found to be 1 ng/ml.

Prostate cancer detection using urine as a non invasive method was examined by spiking urine sample with different PSA buffer concentrations. A negative control (No PSA protease) was tested to ensure that the MNPs-peptide moieties dissociation was attributed to PSA proteolytic activity (Fig 2 B). LOD was found to be 0.5 ng/ml. The designed biosensor showed adequate long-term stability for up to three months at room temperature.

4 Conclusion

The present study demonstrated the ability of the developed biosensor to detect PSA semi-quantitatively by naked eye. Additionally, this biosensor provides very simple protease detection tool without washing and/or blocking steps. This unique advantage is crucial for the development of cost-effective, lab-on-a-chip device suitable for point-of-care usage.

Acknowledgements:

The authors would like to acknowledge the financial support from The University of Jordan.

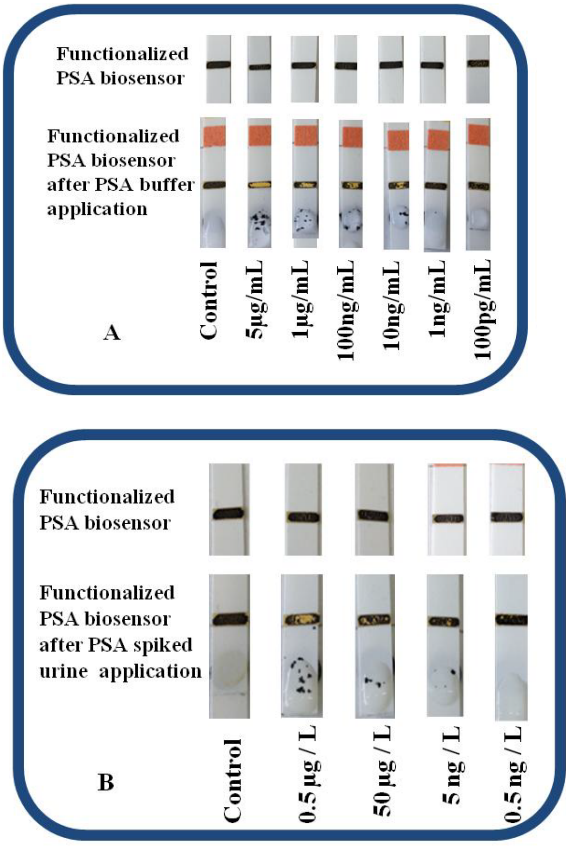

Fig. 2. Biosensing of different concentrations of PSA in buffer (A) and in spiked urine (B).

References:

[1] Neurath, H. and K.A. Walsh, Role of proteolytic enzymes in biological regulation (a review). Proceedings of the National Academy of Sciences, 73,11, 1976, pp. 3825-3832.

[2] Woodward, J.K.L., et al., The roles of proteolytic enzymes in the development of tumour-induced bone disease in breast and prostate cancer. Bone, 41, 6, 2007, pp. 912-927.

[3] Suaifan, G.A., et al., Proteinases As Biomarkers In Breast Cancer Prognosis And Diagnosis. Mini Reviews in Medicinal Chemistry, in press, 2016.

[4] Suaifan, G.A., S. Alhogail, and M. Zourob, Rapid and low-cost biosensor for the

detection of Staphylococcus aureus. Biosensors and Bioelectronics, 90, 2017, pp. 230-237.
[5] Suaifan, G.A., S. Alhogail, and M. Zourob, Paper-based magnetic nanoparticle-peptide probe for rapid and quantitative colorimetric detection of Escherichia coli O157:H7. Biosensors and Bioelectronics, in press, 2016.
[6] Alhogail, S., G.A. Suaifan, and M. Zourob, Rapid colorimetric sensing platform for the detection of Listeria monocytogenes foodborne pathogen. Biosensors and Bioelectronics, 86, 2016, pp. 1061-6.
[7] Wignarajah, S., et al., Colorimetric Assay for the Detection of Typical Biomarkers for Periodontitis Using a Magnetic Nanoparticle Biosensor. Analytical Chemistry, 87, 24, 2015, pp. 12161-8.
[8] Sun C Fau - Su, K.-H., et al., Time-resolved single-step protease activity quantification using nanoplasmonic resonator sensors. ACS Nano, 4, 2, 2010, pp. 978-984.
[9] Wu, P., et al., Immunopeptidometric Assay for Enzymatically Active Prostate-Specific Antigen. Clinical Chemistry, 50, 1, 2004, pp. 125-129.
[10] Denmeade, S.R., et al., Specific and Efficient Peptide Substrates for Assaying the Proteolytic Activity of Prostate-specific Antigen. Cancer Research, 57, 21, 1997. pp. 4924-4930.
[11] Suaifan, G.A., et al., Recent progress in prostate-specific antigen and HIV proteases detection. Expert Reviews in Molecular Diagnostics, 13, 7, 2013, pp. 707-18.
[12] Suaifan, G.A., et al., Wash-less and highly sensitive assay for prostate specific antigen detection. Analyst, 137, 23, 2012, pp. 5614-5619.
[13] Esseghaier, C., et al., One-step assay for optical prostate specific antigen detection using magnetically engineered responsive thin film. Journal of Biomedical Nanotechnology, 10, 6, 2014, pp. 1123-9.
[14] Feng, T., et al., A graphene oxide-peptide fluorescence sensor for proteolytically active prostate-specific antigen, in Molecular BioSystems. The Royal Society of Chemistry, 2012, pp. 1441-1445.
[15] Raj, G.V., A.W. Partin, and T.J. Polascik, Clinical utility of indium 111-capromab pendetide immunoscintigraphy in the detection of early, recurrent prostate carcinoma after radical prostatectomy. Cancer, 94, 4, 2002, pp. 987-996.
[16] Sengupta, S., et al., Prostate Specific Antigen Kinetics in the Management of Prostate Cancer. The Journal of urology, 179, 3, 2008, pp. 821-826.
[17] Vessella, R.L., J. Noteboom, and P.H. Lange, Evaluation of the Abbott IMx automated immunoassay of prostate-specific antigen. Clinical Chemistry, 38, 10, 1992, pp. 2044-54.
[18] Junker, R., et al., Comparison of prostate-specific antigen (PSA) measured by four combinations of free PSA and total PSA assays. Clinical Chemistry, 43, 9, 1997, pp. 1588-1594.