

# An Immunosensor for Bladder Cancer Screening

Congo Tak Shing Ching, Hong-Sheng Chen, Tai-Ping Sun and Hsiu-Li Shieh

**Abstract**—Nuclear matrix protein 22 (NMP22) is a FDA approved biomarker for bladder cancer. The objective of this study is to develop a simple NMP22 immuosensor (NMP22-IMS) for accurate measurement of NMP22. The NMP22-IMS was constructed with NMP22 antibody immobilized on screen-printed carbon electrodes. The construction procedures and antibody immobilization are simple. Results showed that the NMP22-IMS has an excellent ( $r^2 \geq 0.95$ ) response range (20 – 100 ng/mL). In conclusion, a simple and reliable NMP22-IMS was developed, capable of precisely determining urine NMP22 level.

**Keywords**—Bladder Cancer, Immunosensor, Impedance, NMP22

## I. INTRODUCTION

BLADDER cancer is a global health problem. It held fourteenth and eleventh position of the leading causes of cancer death in Taiwan in 2009<sup>[1]</sup> and in the whole world in 2008<sup>[2]</sup>, respectively.

Traditional diagnostic methods for bladder cancer include urine cytology test, bladder endoscopic diagnosis, bladder ultrasonic diagnosis and etc. However, these methods are inconvenience. NMP22 is a FDA approved biomarker for bladder cancer. Regular monitoring of urine NMP22 can decrease the incidence or mortality rate for the people who are at the high risk of bladder cancer. Nevertheless, routine urine NMP22 level can only be assayed in hospital or clinic but not at home. Although there is a published NMP22 biosensor paper, it is complicated in its fabrication procedures<sup>[3]</sup>. Hence, this study aims to build up a NMP22-IMS for accurate measurement of urine NMP22 level.

## II. MATERIALS AND METHODS

### A. Reagents and Solutions

Commercial reagents were used in this study with no additional purification. Phosphate-buffered saline (PBS), bovine serum albumin (BSA) and glutaraldehyde were bought from Sigma Chemical Company (St Louis, MO). NMP22 antigen and antibody were bought from AbKing Biotechnology Co. Ltd. (Taipei, Taiwan). Deionized water (resistivity  $\geq 18$  M $\Omega$ cm), purified by a Millipore Milli-Q UFplus System (Bedford, MA), was used for all solutions preparation.

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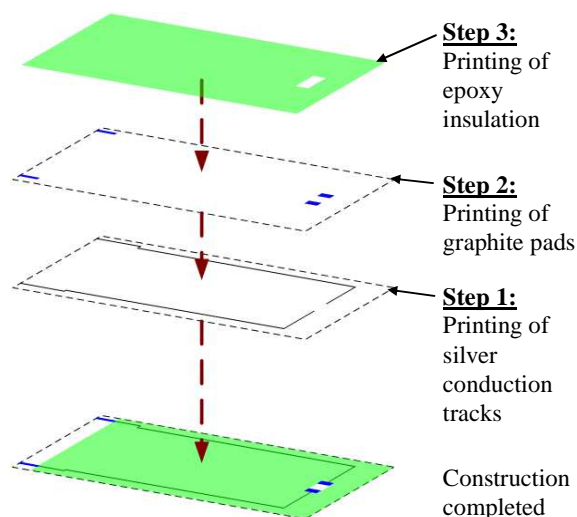


Fig. 1 The screen printing construction steps for the NMP22-IMS. Polyethylene terephthalate is used as the substrate for the immuosensor. After each layer screen printing, it is placed at 80 °C for 30 minutes with the aim of drying the layer before the next layer screen printing

### B. Equipment

An impedance analyzer (Precision Impedance Analyzer WK6420C, Wayne Kerr Electronics Ltd, UK) was used for all impedance spectrum measurements.

### C. NMP22-IMS Construction

Sensor was constructed by screen printing technique. The screen mesh size and screen emulsion thickness was equal to 390 counts per inch and 25  $\mu$ m, respectively. The construction steps were schematically shown in Fig. 1. There were 3 different screen printing layers for each sensor. The first, second and third layers was the silver conducting tracks, graphite pads and epoxy insulating shroud, respectively. Polyethylene terephthalate sheet was used for the substrate of the sensor. After each layer screen printing, it was placed at 80 °C for 30 minutes in order to dry the layer before the next layer screen printing.

For NMP22 antibody immobilization, glutaraldehyde (2.5%, 4  $\mu$ L) was dropped onto the graphite pads of the sensor. Subsequently, a mixture of NMP22 antibody (100 ng/mL, 2  $\mu$ L) and BSA (0.1 M, 1  $\mu$ L) was dropped again onto the graphite pads of the sensor and kept at 4 °C overnight.

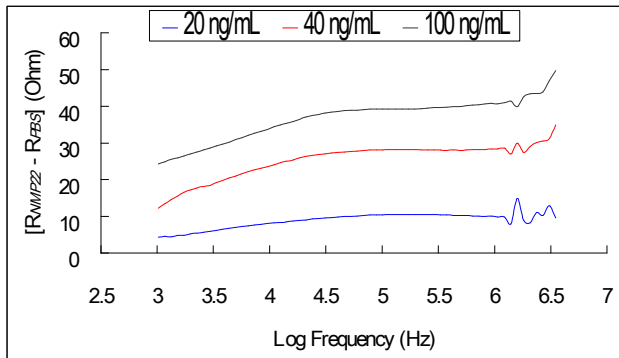


Fig. 2 The real part of impedance response of the NMP22-IMS to NMP22 at various concentrations (20, 40 and 100 ng/mL) within the frequency range of 1 kHz – 3.5 MHz. An excellent linear response ( $r^2 \geq 0.95$ ) was observed within these frequency range

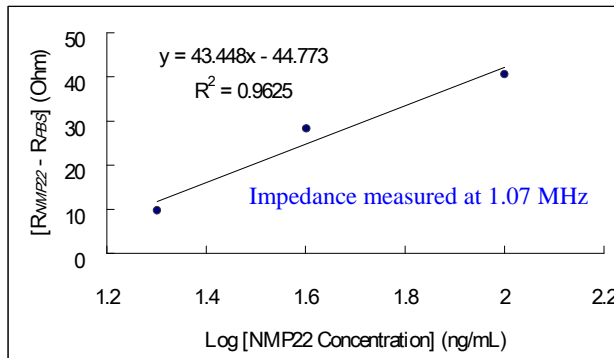


Fig. 3 Calibration curve of the NMP22-IMS on measuring NMP22 (20 – 400 ng/mL) at 1.07 MHz. The NMP22-IMS has excellent linear response, with  $r^2=0.96$  and sensitivity of 43.45  $\Omega/\text{Log}(\text{ng/mL})$

**D. Measurements of the Real Part of Impedance Response of the NMP22-IMS to NMP22**

All impedance spectrum measurements were carried out at room temperature and recorded over the frequency range of 1 kHz – 3.5 MHz. There was 100 frequency points per logarithmic decade within this frequency range. The amplitude of the perturbing wave was limited to 100 mV.

To carry out the measurement, the NMP22-IMS was connected to the impedance analyzer. PBS (10  $\mu\text{L}$ , 25 mM, pH 7.0) was pipetted onto the NMP22-IMS. After waiting for 60 seconds, real part of the impedance spectrum of the PBS ( $R_{\text{PBS}}$ ) was then recorded.

After that, the PBS was removed and 10  $\mu\text{L}$  NMP22 (20, 40 and 100 ng/mL) was subsequently pipetted onto the NMP22-IMS. After waiting for 180 seconds, the NMP22 was then removed and the NMP22-IMS was immersed and softly washed with PBS (25 mM, pH 7.0). Then, a fresh PBS (10  $\mu\text{L}$ , 25 mM, pH 7.0) was consequently pipetted onto the NMP22-IMS. After waiting for 60 seconds, real part of the impedance spectrum was recorded again and it is called real part of the impedance spectrum of the NMP22 ( $R_{\text{NMP22}}$ ). The real part of impedance response of the NMP22-IMS to NMP22 is calculated by subtracting  $R_{\text{NMP22}}$  from  $R_{\text{PBS}}$  (i.e.  $R_{\text{NMP22}} - R_{\text{PBS}}$ ) of NMP22 at various concentrations.

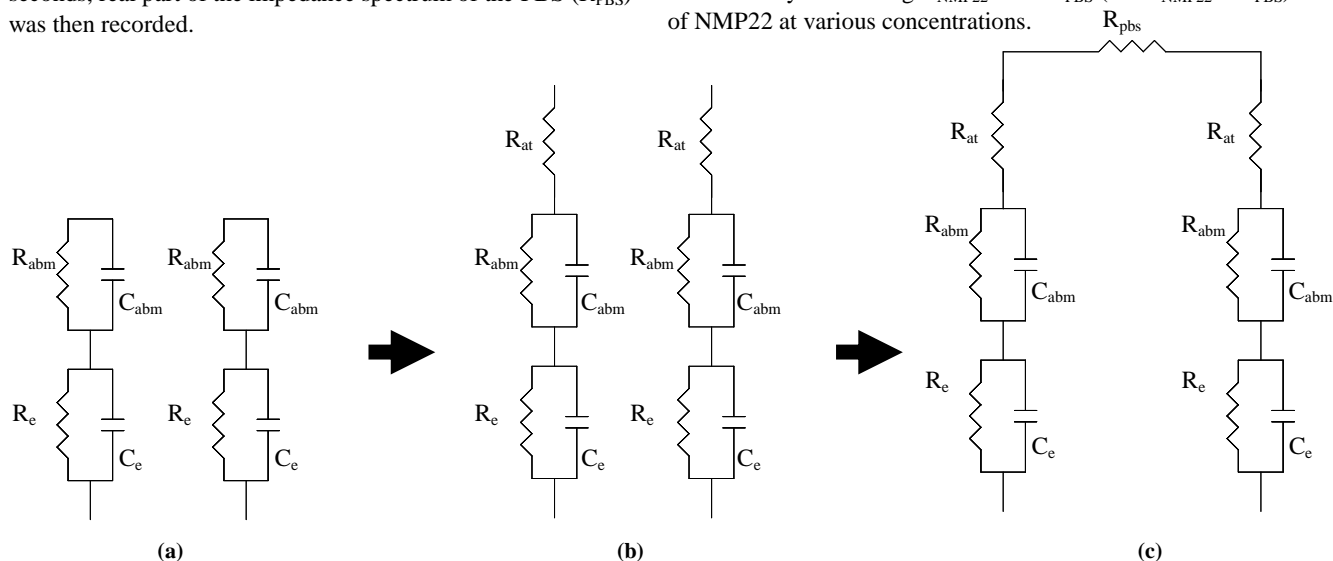


Fig. 4 (a) An equivalent circuit represents the NMP22-IMS, with  $R_e$  and  $C_e$  representing the resistance and capacitance of the graphite pads of the NMP22-IMS, respectively.  $R_{\text{abm}}$  and  $C_{\text{abm}}$  represent the resistance and capacitance of the antibody mixture (i.e. glutaraldehyde + BSA + NMP22 antibody) immobilized on top of the graphite pads, respectively. (b) An equivalent circuit represents the NMP22-IMS combining with the NMP22 antigen, with  $R_{\text{at}}$  representing the resistance of the NMP22 antigen. (c) An equivalent circuit represents the NMP22-IMS (after the combination with NMP22 antigen) under impedance measurements at the medium of PBS solution, with  $R_{\text{pbs}}$  representing the resistance of the PBS solution.

### III. RESULTS AND DISCUSSION

Fig. 2 showed the subtracted real part of impedance spectrum (i.e.  $R_{NMP22} - R_{PBS}$ ) of NMP22 at various concentrations (20 – 100 ng/mL) within the frequency range of 1 kHz – 3.5 MHz. An excellent linear response ( $r^2 \geq 0.95$ ) was observed within these frequency range. This excellent linear response range covers the pathological ranges of urine NMP22 levels. Fig. 3 showed the calibration curve of the NMP22-IMS on measuring NMP22 at 1.07 MHz. It was found that the NMP22-IMS has excellent linear ( $r^2 = 0.95$ ) response with the sensitivity of 43.45  $\Omega/\text{Log}(\text{ng/mL})$ .

As shown in Fig. 2, the increase of NMP22 concentration results in the increase of the subtracted real part of impedance response (i.e.  $R_{NMP22} - R_{PBS}$ ). A model (see Fig. 4) is therefore developed. As shown in Fig 4c, impedance measurement at high frequency results in all capacitors becoming as all conductors. Therefore, all resistors in Fig. 4c are connected in series. Since the resistance of the graphite pads ( $R_e$ ), antibody mixture ( $R_{abm}$ ) and PBS solution ( $R_{pbs}$ ) is constant, the real part of impedance response is therefore governed by the resistance of the NMP22 antigen. As a results, the increase of NMP22 concentration results in the increase of the real part of impedance response (see Fig. 2).

### IV. CONCLUSION

A simple and reliable NMP22 immuosensor was successfully designed and developed. It has an excellent linear response with  $r^2 \geq 0.95$ . The NMP22 immuosensor has a linear working range, 20 – 100 ng/mL. It is capable of precisely determining urine NMP22 levels in the range of pathological region. Therefore, a screening method was proposed in this study with the advantages of rapidity and inexpensiveness.

### ACKNOWLEDGMENT

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