

Colorectal Cancer Screening by a CEACAM-6 Immunosensor

C. T. S. Ching, P. W. Chen, T. P. Sun, and H. L. Shieh

Abstract—The biomarker for colorectal cancer (CRC) is CEACAM-6 antigen (C6AG). Therefore, this study aims to develop a novel, simple and low-cost C6AG immunosensor (C6AG-IMS), based on electrical impedance measurement, for precise determination of C6AG. A screen-printed graphite electrode was constructed and used as the sensor, with CEACAM-6 antibody (C6AB) immobilized on it. The procedures of sensor fabrication and antibody immobilization are simple and low-cost. Impedance measurement at a definite frequency ranges (0.43 – 1.26 MHz) showed that the C6AG-IMS has an excellent linear ($r^2 > 0.9$) response range (8.125 – 65 pg/mL), covering the normal physiological and pathological ranges of blood C6AG levels. Also, the C6AG-IMS has excellent reliability and validity, with the intraclass correlation coefficient of 0.97. In conclusion, a novel, simple, low-cost and reliable C6AG-IMS was designed and developed. The C6AG-IMS can provide a point-of-care and immediate screening results to the user at home.

Keywords—Colorectal Cancer, Immunosensor, Electrical Impedance, CEACAM-6

I. INTRODUCTION

COLORECTAL cancer (CRC) is a global health problem and it held the fourth position of the leading causes of cancer death worldwide [1]. In Taiwan, CRC has been raising from the fifth to the third position of the leading causes of cancer death from 1981 to 2010 [2]. According to the market research conducted by Kalorama Information, 41 million US dollars has been spent on the CRC diagnosis in the USA in 2007. Therefore, the market for the CRC diagnosis is massive [3]. Importantly, CRC diagnosis in Taiwan is not included in the paid-item of the National Health Insurance.

In Taiwan, CRC diagnostic methods include digital rectal examination (DRE), occult blood stool, sigmoidoscopy (SMDS), colonoscopy (CS), barium enema, and etc [4]. However, these methods definitely cause patient discomfort and unwillingness to take the test. Moreover, no matter DRE, SMDS or CS is used, colorectal cleaning and insertion diagnosis are necessary, and this may make patients embarrassing.

Researches showed that CEACAM-6 antigen (C6AG) is a biomarker for CRC screening [5]. To the best of our knowledge, there are no sensors for C6AG measurement being reported in the literatures. Hence, a CEACAM-6 antigen immunosensor (C6AG-IMS) was designed and developed in this study for CRC screening. This immunosensor can provide

a point-of-care and immediate screening results to the user at home.

II. MATERIALS AND METHODS

A. Reagents and Solutions

In this study, commercial reagents were used without further purification. Bovine serum albumin (BSA), glutaraldehyde, phosphate-buffered saline (PBS) were purchased from Sigma Chemical Company (St Louis, MO). C6AB and C6AG were purchased from Abnova (Taipei, Taiwan). Deionized water, with the resistivity greater than 18 MΩcm, was used for all solutions preparation and it was purified by a Millipore Milli-Q UFplus System (Bedford, MA).

B. Equipment

All electrical impedance spectrum measurements were conducted by the use of an impedance analyzer (Precision Impedance Analyzer WK6420C, Wayne Kerr Electronics Ltd, UK).

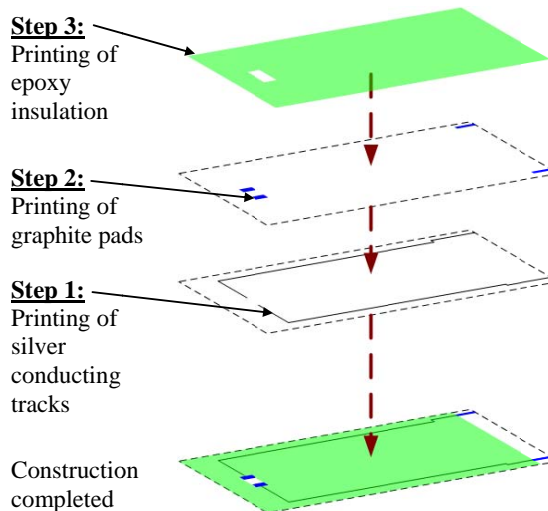


Fig. 1 The procedures of screen printing construction for the sensor. The sensor substrate is polyethylene terephthalate. The sensor is placed at 80 °C for 0.5 hour after each layer screen printing. The intention of this is to dry the layer before the next layer screen printing

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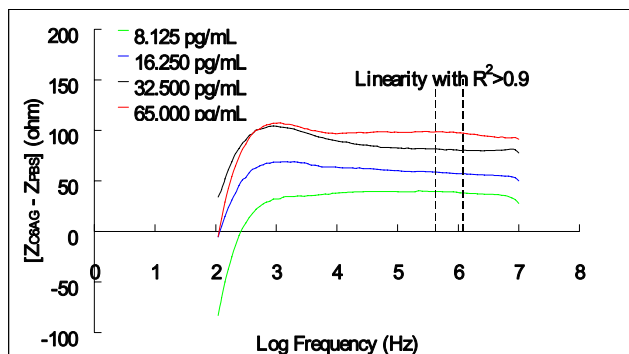


Fig. 2 The C6AG-IMS impedance response to C6AG at various concentrations (8.125 – 65 pg/mL) within the frequency range of 110 Hz – 10 MHz. At a definite frequency range (0.43 – 1.26 MHz), an excellent linear response range (8.125 – 65 pg/mL), with $r^2 > 0.9$, was observed

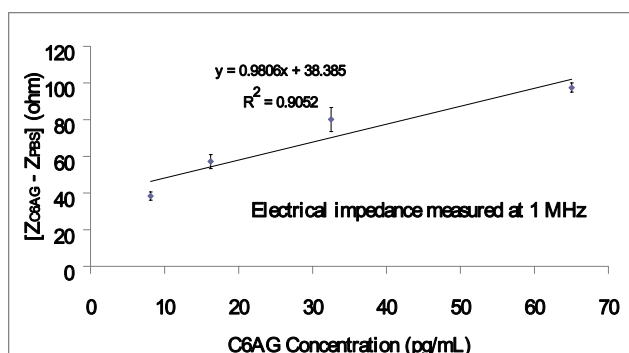


Fig. 3 Calibration curve of the C6AG-IMS on measuring C6AG (8.125 – 65 pg/mL) at 1 MHz, within the definite frequency range (0.43 – 1.26 MHz). The C6AG-IMS has excellent linear response, with $r^2 = 0.9$ and sensitivity of $0.98 \Omega / (\text{pg/mL})$

insulating shroud. The substrate of the sensor was polyethylene terephthalate sheet. After each layer screen printing, it was placed at 80 °C for 0.5 hour with the purpose of dry the layer before the next layer screen printing.

For C6AB immobilization, glutaraldehyde (2.5%, 4 μL) was firstly pipetted onto the graphite pads of the sensor. Subsequently, a mixture of C6AB (1 $\mu\text{g/mL}$, 2 μL) and BSA (0.1 M, 1 μL) was pipetted again onto the graphite pads of the sensor. Finally, the sensor was kept at 4 °C overnight.

D. Measurements of the Impedance Response of the C6AG-IMS to C6AG

Electrical impedance spectrum measurements were recorded over the frequency range of 110 Hz – 10 MHz. All measurements were conducted in the condition of room temperature. Within this frequency range, there was 100 frequency points per logarithmic decade. The amplitude of the perturbing wave was limited to 100 mV.

To perform the electrical impedance spectrum measurement, the C6AG-IMS was connected to the impedance analyzer. PBS (10 μL , 25 mM, pH 7.0) was firstly pipetted onto the C6AG-IMS. One minute later, the electrical impedance spectrum of the PBS (Z_{PBS}) was recorded. Then, the PBS was removed and 10 μL of C6AG (8.125, 16.25, 32.5 and 65 pg/mL) was subsequently pipetted onto the C6AG-IMS. Three minutes later, the C6AG was then removed and the C6AG-IMS was immersed and softly washed with PBS (25 mM, pH 7.0). Then, a fresh PBS (10 μL , 25 mM, pH 7.0) was consequently pipetted onto the C6AG-IMS. One minute later, the electrical impedance spectrum was recorded again and it is called the electrical impedance spectrum of the C6AG (Z_{C6AG}). The electrical impedance response of the C6AG-IMS to C6AG is calculated by subtracting Z_{C6AG} from Z_{PBS} (i.e. $Z_{\text{C6AG}} - Z_{\text{PBS}}$) of C6AG at various concentrations.

III. RESULTS AND DISCUSSION

Fig. 2 showed the subtracted impedance spectrum (i.e. $Z_{\text{C6AG}} - Z_{\text{PBS}}$) of C6AG at various concentrations within the frequency range of 110 Hz – 10 MHz. A definite frequency range (0.43 – 1.26 MHz) was found, in which an excellent linear response range (8.125 – 65 pg/mL) could be obtained, with $r^2 > 0.9$. This excellent linear response range covers the normal physiological and pathological ranges of blood C6AG levels. Fig. 3 showed the calibration curve of the C6AG-IMS on measuring C6AG at the measurement frequency of 1 MHz. It was found that the C6AG-IMS has excellent linear ($r^2 = 0.9$) response with the sensitivity of $0.98 \Omega / (\text{pg/mL})$.

The reproducibility in the construction of the C6AG-IMS was evaluated from standard deviation of the subtracted impedance spectrum (i.e. $Z_{\text{C6AG}} - Z_{\text{PBS}}$). As shown in Table I, the relative standard deviation of the subtracted impedance spectrum indicated a batch reproducibility of C6AG-IMS construction of about 8.1%.

Intraclass correlation coefficient (ICC) can be used to evaluated the reproducibility of a variable. In general, $\text{ICC} > 0.75$ is suggested for good reliability [6]. However, in

TABLE I
EVALUATION OF THE C6AG-IMS ON THE DETERMINATION OF THE CONCENTRATIONS OF C6AG SOLUTIONS AT THE MEASUREMENT FREQUENCY OF 1MHZ

Standard C6AG Solutions (pg/mL)	Subtracted Impedance Spectrum (Ω), i.e. $Z_{\text{C6AG}} - Z_{\text{PBS}}$			Intraclass Reliability (ICC 3,1)
	Mean (Ω)	SD (Ω)	Relative SD (%)	
8.125	38.3	2.2	5.7	0.97
16.25	57.15	3.75	6.6	
32.5	80.1	6.5	8.1	
65	97.5	2.5	2.6	

C. C6AG-IMS Construction

Sensor was constructed by screen printing technique. The screen mesh size was 390 counts per inch and screen emulsion thickness was 25 μm . Fig. 1 schematically shows the fabrication steps. Each sensor has 3 different screen printing layers. The first layer was silver conducting tracks. The second layer was graphite pads. And, the third layer was epoxy

medical measurements, ICC>0.90 is definitely required in order to guarantee reasonable validity [7]. The ICC(3,1) value was 0.97 in this study (Table I). This suggests that they have excellent reliability and validity.

IV. CONCLUSION

A novel, simple, low-cost and reliable C6AG-IMS was successfully designed and developed. It has an excellent linear response with $r^2>0.9$. The C6AG-IMS has a linear working range, 8.125 – 65 pg/mL. It is capable of precisely determining C6AG levels in the range of normal physiological and pathological region. Therefore, a screening method was proposed in this study with the advantages of rapidity and inexpensiveness. Also, the C6AG-IMS can provide a point-of-care and immediate screening results to the user at home.

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