

Enzymeless Osteopontin Biosensor Based on Impedance Measurement

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Abstract—In developed countries, ovarian cancer has become one of the main mortal cancers in gynecopathy. Like in America, it is estimated that one out of seventy women have suffered from ovarian cancer. Therefore, the aim of this study is to develop an enzymeless, simple, low-cost and reliable osteopontin biosensor (OBS) for the precise determination of osteopontin. The OBS is a bowl cell and it is consisted of 4 silver electrodes. The working principle of the OBS is based on the impedance measurement of osteopontin solution. At a specific frequency ranges ($96.58\text{--}10 \times 10^6 \text{Hz}$), the OBS on the detection of osteopontin shows an excellent linear ($r^2 > 0.95$) response range ($2500\text{--}75000 \text{ng/dl}$), which covers the normal physiological and pathological ranges of plasma osteopontin levels. Intraclass correlation coefficient showed that all measurements had excellent reliability and validity ($\text{ICC} > 0.90$). In conclusion, an enzymeless, simple, low-cost and reliable OBS was developed. It is capable of precisely determine osteopontin concentrations in both the normal physiological and pathological ranges.

Keywords- Osteopontin; Biosensor; Impedance

I. INTRODUCTION

In developed countries, ovarian cancer has recently become one of the main mortal cancers in gynecopathy. According to the statistics from the Gynecologic Oncology Group, there is approximately 30% five-year survival rate in ovarian cancer. In America, about 21,650 new cases of ovarian cancer were diagnosed in 2008, accounting for about 3 percent of all cancers among women [1]. And, it is estimated that 15,520 women died from ovarian cancer in 2008 and ovarian cancer ranks second among gynecologic cancers [1]. In Taiwan, according to the statistics from the Bureau of Health Promotion, Department of Health, R.O.C., ovarian cancer held the second position among gynecological reproductive cancers, just behind the cervical cancer [2]. Therefore, it would be great if a reliable, quick and inexpensive screening method is available for high-risked people.

Luckily, a biomarker has been found for the ovarian cancer and it is the osteopontin [3-5]. Studies showed that the concentration of plasma osteopontin of ovarian cancer patients is 2-3 times higher than that of healthy people [4]. Therefore, osteopontin biosensor (OBS) has been

investigated in this study. To the best of our knowledge, no OBS have been reported on in the literature (searched by the ScienceDirect, PubMed and MEDLINE search engine using the keyword of osteopontin and biosensor). Therefore, the current study is the first to report an enzymeless approach for osteopontin determination and this is our aim and novelty.

The working principle of the enzymeless OBS is based on the impedance measurement of osteopontin solution. Impedance measurement is obtained by injecting low-level sinusoidal current in the osteopontin solution and measuring the voltage drop generated by the impedance of the osteopontin solution.

The aim of this study is to develop an enzymeless, simple, low-cost and reliable OBS for the precise determination of osteopontin.

II. MATERIALS AND METHODS

A. Chemicals and reagents

Osteopontin and 10X phosphate buffered saline were purchased from Sigma Chemical Co. (St. Louis, MO). De-ionized water purified by a Millipore System (Milli-Q UFplus; Bedford, MA) was used to dilute the 10X phosphate buffered saline so as to obtain a 0.01M phosphate buffered saline (pH 7.4, 0.154M sodium chloride). The 0.01M phosphate buffered saline was then used to prepare osteopontin solutions with various concentrations.

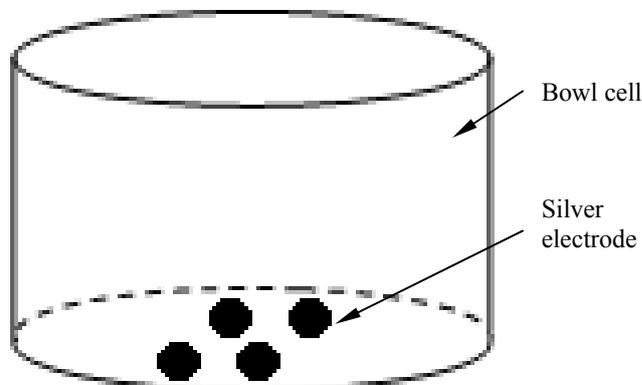


Figure 1. The osteopontin biosensor.

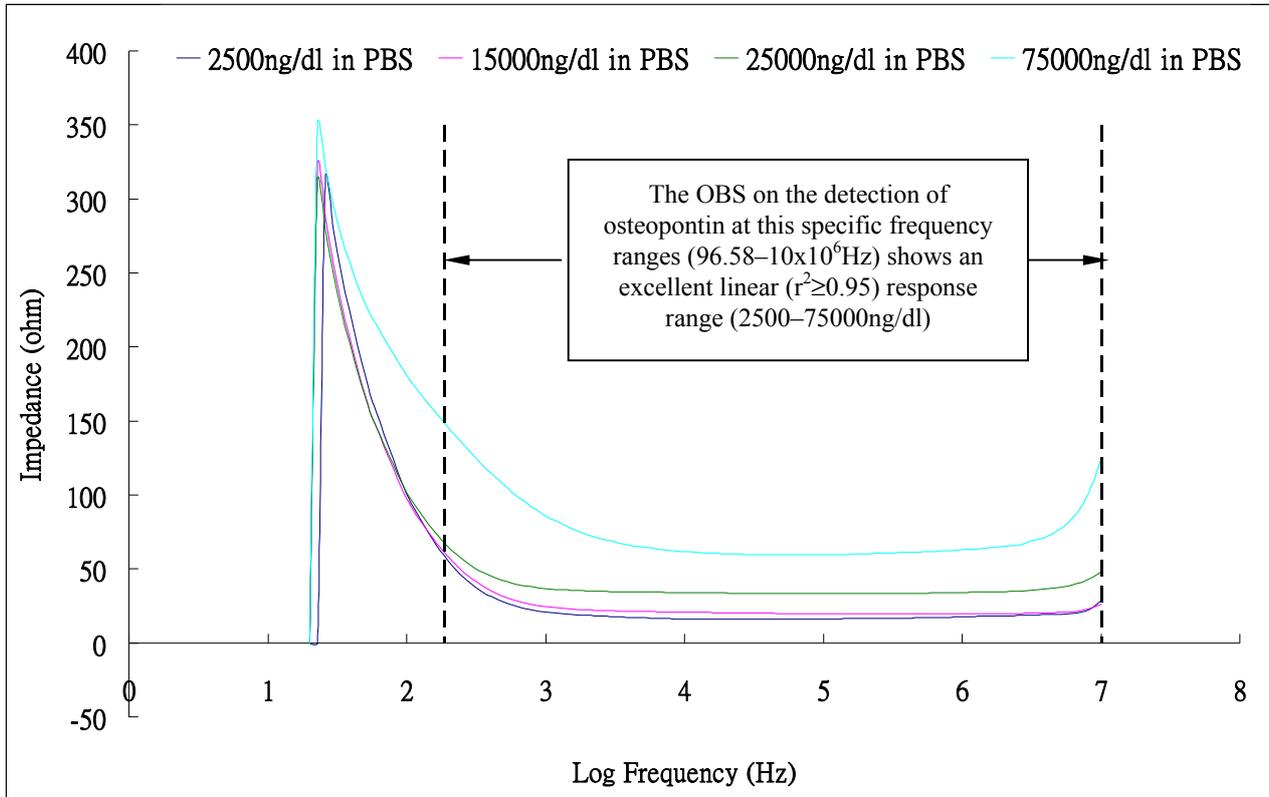


Figure 2. The impedance spectrum of osteopontin solution at various concentrations within the frequency range of $20\text{--}10 \times 10^6 \text{ Hz}$.

B. The enzymeless OBS and its working principle

The OBS is a bowl cell. It was consisted of four 1-mm-diameter silver electrodes (2.5mm between electrode centers) mounted in square configuration (Figure 1). The working principle of the OBS is based on the impedance measurement of osteopontin solution.

C. Impedance spectrum measurement to osteopontin

All impedance spectrum measurements were carried out at room temperature. The OBS was connected to an impedance analyzer (Precision Impedance Analyzer WK6420C, Wayne Kerr Electronics Ltd, United Kingdom). One hundred microliter of osteopontin solution (2500, 15000, 25000, 75000ng/dl) was pipetted onto the OBS. Impedance spectrum of osteopontin solutions was recorded over the frequency range $20\text{--}10 \times 10^6 \text{ Hz}$. Within this frequency range, there was 17 frequency points per logarithmic decade. The amplitude of the perturbing wave was limited to 200mV. In order to check reliability of the measurements, three separate sets of measurement of an osteopontin solution were made in succession.

III. RESULTS AND DISCUSSION

With the intention of obtaining the measured impedance essentially independent of the contact impedance between

the silver electrodes and the osteopontin testing solution, the OBS was consisted of four-electrode configuration.

Figure 2 showed the impedance spectrum of osteopontin solution at various concentrations within the frequency range of $20\text{--}10 \times 10^6 \text{ Hz}$. A specific frequency range ($96.58\text{--}10 \times 10^6 \text{ Hz}$) was found, in which an excellent linear ($r^2 \geq 0.95$) response range (2500–75000ng/dl) could be obtained (Figure 3). This excellent linear response range covers the normal physiological and pathological ranges of plasma osteopontin levels.

The intraclass correlation coefficient (ICC) is a measure used to quantify the reproducibility of a variable. ICC is also a measure of the homogeneity within groups of replicate measurements relative to the total variation between groups. In general, ICC values above 0.75 have been suggested for good reliability while ICC values below 0.75 have been suggested for poor to moderate reliability [6]. For many clinical measurements reliability should exceed 0.90 to ensure reasonable validity [7]. In this study, the ICC(3,1) values were above 0.90 (Table I) and this suggests that they have excellent reliability and validity.

Screen printing technique is very common and therefore the cost and the size can be further reduced.

Research is ongoing to study the interference study, the long-term stability of the OBS and the impedance spectrum of osteopontin in blood. This result will be reported in due course.

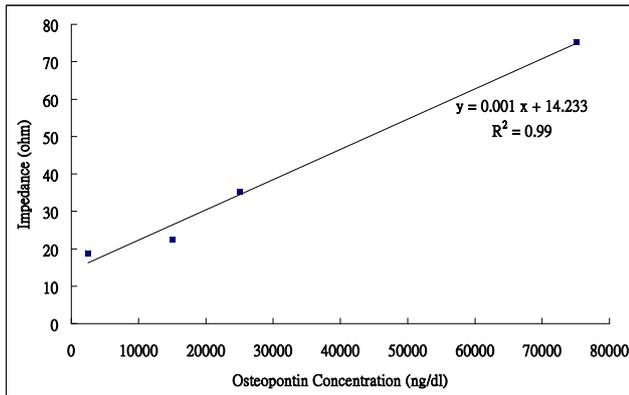


Figure 3. Calibration curve of the impedance measured by the OBS at the frequency of 1732.526Hz, within the frequency range of 96.58–10x10⁶Hz, to the osteopontin concentration (2500–75000ng/dl).

TABLE I. ESTIMATION OF INTRARATER RELIABILITY (ICC3,1) FOR THE MEASUREMENT OF IMPEDANCE OF STANDARD OSTEOPONTIN SOLUTION WITHIN THE FREQUENCY RANGES OF 96.58–10x10⁶Hz.

Standard Osteopontin Solution	ICC 3,1
2500ng/dl	0.99
5000ng/dl	1.00
15000ng/dl	1.00
25000ng/dl	0.98
35000ng/dl	1.00
45000ng/dl	1.00
50000ng/dl	1.00
65000ng/dl	1.00
75000ng/dl	0.92

IV. CONCLUSION

A novel enzymeless osteopontin biosensor (OBS) was successfully designed and developed. The OBS is highly-sensitive, reliable, simple and low-cost. A specific frequency range (96.58–10x10⁶Hz) was found for the impedance measurement of osteopontin by the OBS, in which an excellent linear response range could be obtained. The linear working range (2500–75000ng/dl) of the OBS is able to determine the normal physiological and pathological osteopontin plasma levels. Therefore, this allows a quick and inexpensive screening method.

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