

Impedimetric Point-of-Care Cardiac Marker System

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Abstract

The requirement for fast identification, with accurate and reliable diagnostics for acute myocardial infarction or “Heart Attack” has generated what can be called “Point-of-Care (POC)” devices.

This paper will use key engineering platforms developed that can address the need for improved clinical management in cardiology and reduce Health Service delivery costs. Our primary objective is to develop rapid and label-free Point of Care diagnostic tests for cardiac markers based on multi-frequency impedimetric transducers.

Impedance testing using micro-scale interdigitated electrodes (μIDE 's) offers a much more rapid response compared to the reaction times of fluorescent label-based systems (~ 8 minutes). In the case of small gap of IDE impedance, we are seeing stable and predictive trends occurring after one minute. Therefore the overall benefit to the clinician and patient will be true point of care monitoring allowing a blood sample to be rapidly (1-2 minutes) analysed for a range of cardiac enzymes.

1. Introduction

Testing for cardiac enzymes, from blood samples, along with a full patient examination and ECG is a routine part of diagnosing damage to the heart. Enzyme concentration can rise slowly (after 2 hours) and it is often common practice to repeat the test on an ongoing basis. Test results are normally available within 1 – 2 hours. ECG results show High specificity, Low sensitivity and are inconclusive with unreliable results in 50% of all cases.

There is however compelling evidence, highlighted in a number of important reviews, that the management of AMI and ACS is extremely time critical [1-6]. Early data from the US National Heart Attack Alert Program have shown clearly that if therapy is delayed for more than 2 hours then the treatment benefit to patients decreases tremendously (see figure 1.)

According to current guidelines, the results of the

cardiac marker testing should be available to the physician within 30 minutes (“vein-to-brain” time) to initiate therapy within 60–90 minutes (“door-to-needle” time) after the patient has arrived at the emergency room or intensive care unit [1]. It is therefore very clear that the development of point-of-care-testing for cardiac enzymes is highly desirable.

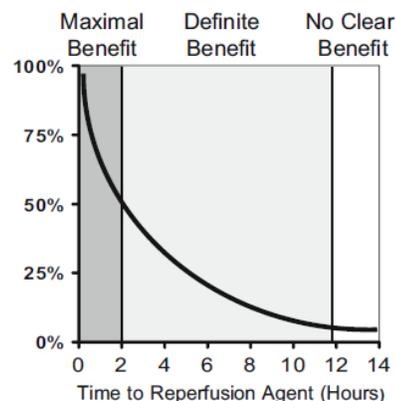


Figure 1. Illustration of treatment benefit after AMI

The development of impedimetric IDE immunosensor for monitoring cardiac enzymes can provide:

- 1- Faster feedback (1-2 minutes) to the clinical team than current STAT lab based diagnostics (1-2 hours) therefore allowing clinicians to maximise patient benefit and reduce costs.
- 2- Improved management of the patient within ambulance; triage; A&E; bed-side; and home due to immediate feedback on overall trends of the cardiac enzyme profiles.
- 3- Evidence based and timely decision making for improved condition management due to enhanced data frequency and accuracy, hence improved pattern recognition and trend analysis capability.

The development of μIDE arrays for multi-frequency impedance measurement offers significant advantages that include enlargement of the surface area, reduced

double layer capacitance, better detection limits and increased signal-to-noise ratios.

Furthermore, μ IDE arrays offer enhanced current densities that are attributed to the increased mass transport at the working electrode interface. Electric field modelling of such arrays has indicated that, as dimensions are reduced, the electric field penetration is restricted to the interface region rather than the bulk electrolyte, thus enhanced sensitivity to binding events is likely [7].

The signal enhancement for micro-scale IDEs is due to smaller diffusion lengths and this has to be further correlated against a range of gap sizes. The small dimensions lead to a high current density at the electrode surface, allowing the study of molecular interactions, fast heterogeneous electron transfer kinetics, and mass transport in the nanometer regime [8].

2. Impedimetric PoC

The proposed miniaturised impedimetric PoC cardiac marker system will consist of appropriate microfluidics system that will be incorporated to provide filtration of the blood cells, μ IDE impedance based transducer to provide cardiac enzymes monitoring and the integration of telemetry, microprocessors and embedded software to allow a complete ‘Lab-on-chip’ approach with smart analysis as illustrated in figure 2.

The miniaturised device will achieve the ultimate goal of blood cardiac enzymes monitoring.

In this paper we are more conenrn on the enhancement of the impedance signal using μ IDEs.

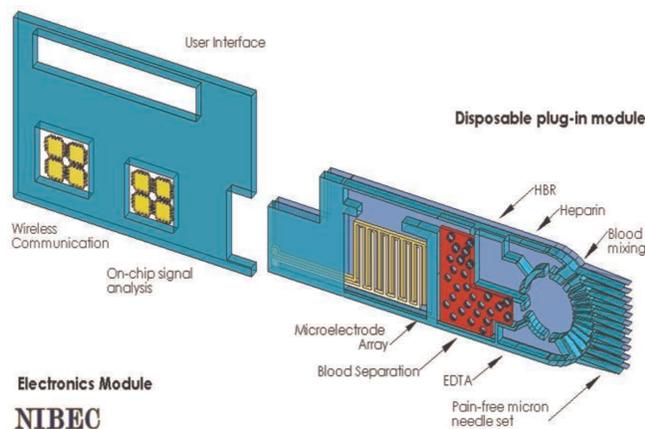


Figure 2. Impedimetric PoC cardiac marker system

2.1. μ IDEs

The IDEs used in this study were fabricated at the Tyndall Institute-Ireland as a part of the National Access Programme (NAP). Their fabrication was achieved using e-beam lithography and a lift-off technique [9][491 Samarao, A.K. 2007] on Pyrex wafers.

The thickness of the Au was maintained at 100 nm with an additional 10 nm Ti adhesion layer. The Au IDEs were flipped face down onto a printed circuit board (PCB). Figure 3. illustrates the packaged chip and depicts the IDES design. The finger width (W) was kept constant at 5 μ m; the gap-size (S) was 10, 7.5, 5 and 2.5 μ m respectively.

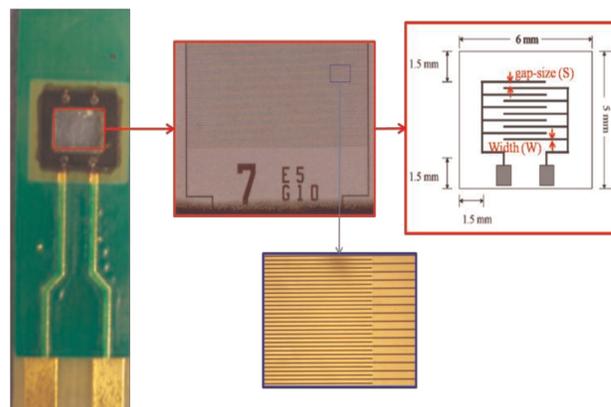


Figure 3. Illustrations of the packaged chip of IDE sensor on PCB with a schematic diagram of IDE configuration

2.2. Electrochemical Impedance Spectroscopy (EIS)

AC impedance spectra were recorded using a Solartron 1260 impedance gain-phase analyzer with a Solartron 1286 electrochemical interface (Solartron Analytical, UK). EIS measurements were performed on all IDEs in a 5 mM $[\text{Fe}(\text{CN})_6]^{3-/4-}$ solution with PBS (phosphate buffer saline) (Sigma, UK).

The Solartron provided an output signal of known amplitude (10 mV) over a frequency range of 1 MHz to 0.1 Hz. Single frequency measurements were also recorded at 130 Hz. The impedance spectra were analysed and fitted to equivalent circuit models using ZPlot® and ZView (Scribner Associates Inc., USA).

2.3. Preparation of IDE immunosensor

The interdigitated electrode was made off gold, in order to immobilise antibodies to gold surface, self-assembled monolayer (SAM) of alkanethiol can provide covalent bonding. Particularly, SAM has some functional groups which are not essential for enzyme, so these groups can be used to provide a covalent attachment with the gold surface and the other end of the alkanethiol is functionalized as to react with antibodies.

In this study, a solution of alkanethiol consists of a mixed of equal volumes of 4mM of mercapto-1-undecanoic acid (MUA) and 1mM of 11-mercaptoundecanoal (MU) solutions prepared in Ethanol,

was exposed to the IDE sensor surface overnight and washed with ethanol to remove unbound thiols.

The other end of thiol was activated using EDC/NHS for 60 min. followed by immobilisation of human Anti myoglobin (MyAb) (500 µg/mL) (HyTest, Finland) for 30 min. The electrode was then rinsed with PBS and unbound IDE sites blocked and cross terminated using 1-Ethanolamine:HCl for 7 min.

Human Myoglobin (MyAg) (HyTest, Finland) was prepared with a range of concentrations (50 ng/mL) in PBS with Tween20. These antigens were injected onto the prepared immunosensor and the change in impedance (ΔZ) measured as a function of time, over the range 50-300 ng/mL.

The change of impedance means the difference in the impedance before and after adding antigens, the change of impedance is denoted by (ΔZ), however it can be calculated from the following equation.

$$\Delta Z = Z_{Ab-Ag} - Z_{Ab}$$

Where Z_{Ab-Ag} is the magnitude of impedance after myoglobin antigen binding to myoglobin antibody, Z_{Ab} is the magnitude of impedance of myoglobin antibodies immobilised onto surface of IDE immunosensor.

3. Results and discussion

In this work, we use interdigitated electrodes (μ IDEs) as EIS platform for monitoring the cardiac enzyme in real-time, our results showed evidences of detection within less than 2 minutes. This detection based on antibody-antigen interaction onto the μ IDE's surface.

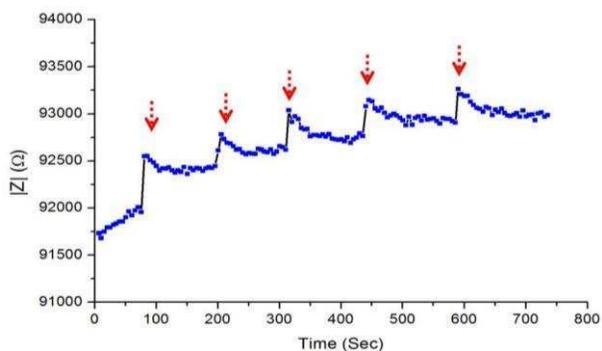


Figure 4. Represents Ab-Ag interactions demonstrated in the change of impedance at 130 Hz versus time; red arrows represent injection of 50 ng/mL of myoglobin at 2 minutes intervals

Figure 4. demonstrates the change of impedance upon the addition of myoglobin antigen to the immunosensor using a single frequency impedance test. In order to verify the antibody-antigen interaction, a multi-frequency

impedance test can be conducted before and after the addition of myoglobin antigens to the IDE immunosensor as illustrated in figure 5.

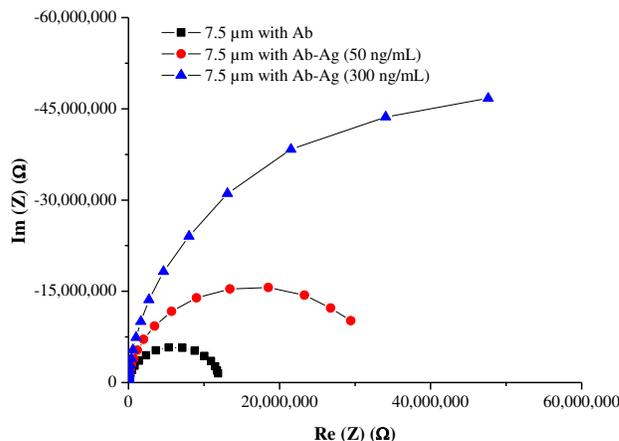


Figure 5. Multi-frequency impedance spectra before and after the addition of myoglobin antigens

Although, the selectivity of the immunosensor was tested by injecting different type of antigen (Rabbit IgG), both types of impedimetric tests (single or multi-frequency) perform no change in the impedance spectra, which confirmed that the IDE immunosensor will provide evidence of response upon the presence of the specific analyte (myoglobin antigen) as illustrated in figure 6.

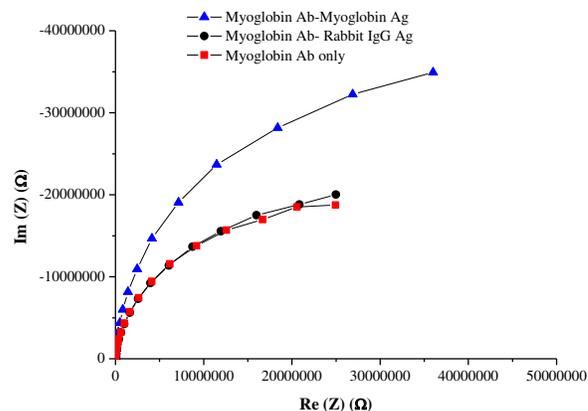


Figure 6. Selectivity test using Rabbit IgG, no change in impedance was obtained after Rabbit IgG, whereas the IDE revealed increasing of impedance upon myoglobin antigens addition

Several single frequency tests using different gap size of IDE immunosensors have been conducted, in order to monitor antibody-antigen interactions for a dynamic range of myoglobin antigens 50-300 ng/mL.

We obtained calibration curves for each IDE immunosensor (as shown in figure 7.). A linear relationship was observed between 50-300 ng/mL, demonstrating sensitivity at levels typically required for

clinical assays.

Table 1. Lists the corresponding change of impedance for each IDE immunosensor with different concentrations of myoglobin antigen.

Table 1. The change of impedance for each IDE immunosensor

Myoglobin (ng/mL)	ΔZ (Ω) S=10 μm	ΔZ (Ω) S=7.5 μm	ΔZ (Ω) S=5 μm	ΔZ (Ω) S=2.5 μm
50	133	146	253	528
100	268	353	388	771
150	407	474	562	999
200	480	600	697	1215
250	661	764	879	1306
300	730	811	1030	1417

These calibration curves revealed a linear relation with respect to the gap-size of the IDE immunosensor, and hence any reduction of the gap-size will increase the sensitivity of IDE immunosensor (as shown in figure 7), due to increasing of the electric field which will induce more charges onto the surface resulting a fast change of the impedance.

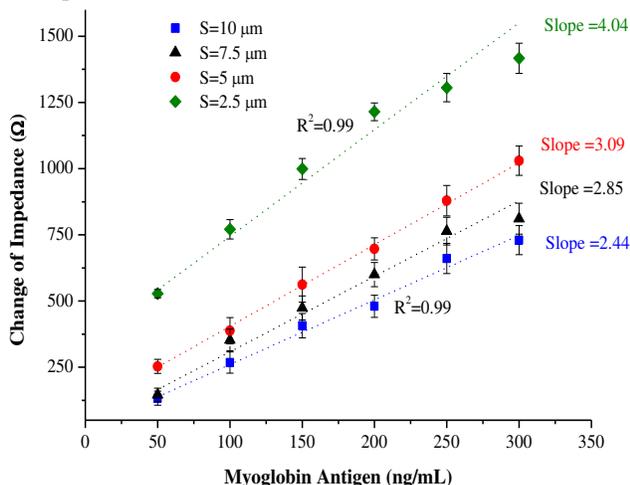


Figure 7. Calibration curve for each IDE as a function of myoglobin Ag concentration

4. Conclusion

In comparison to the work of Ko [10], the EIS immunosensor obtained lower detection (50 ng/mL) limits without the requirement for a labelling antibody.

The system was able to detect small changes of the impedance upon the presence of the cardiac enzymes in less than 1 minute, which can ultimately aid in immediate interventions that can rescue lives and reduce the cost of treatments.

The system will be able to rapidly produce continuous profiles that will significantly aid overall cardiac management. Furthermore production of such devices at low cost (no optics required) and thus opening up potential applications to primary health care and even home based monitoring.

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