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Novel Nanomonitor ultra-sensitive detection of troponin T

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ABSTRACT

Background: Troponin is the preferred biomarker for diagnosing myocardial infarction. Point of care devices have not matched the sensitivity of laboratory-based methods for measuring troponin. The Nanomonitor is a novel point-of-care device that uses the change in electrical impedance that occurs when a biomarker binds to its antibody, which is then correlated to the concentration of the target biomarker.
Methods: Performance characteristics of the Nanomonitor were evaluated and compared to a standard laboratory-based method.
Results: The limit of detection of the Nanomonior for troponin T was 0.0088 ng/l. Total imprecission was 2.38% and 0.85% at troponin T concentrations of 73 ng/l and 1800 ng/l. The functional sensitivity (10% coeffecient of variation) was 0.329 ng/l. The linear regression had a slope of 0.996 (95% confidence interval, 0.991, 1.002), r = 1.00, and an intercept of 15.88 ng/l (95% confidence interval, −68.39 ng/l, 100.15 ng/l). The mean difference between the assays was −7.54 ng/l, determined by Bland–Altman analysis.
Conclusion: The Nanomonitor preliminary results have favorable performance characteristics for detecting troponin T in patient blood, provide results in 15 min, and are portable. More research is needed.
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1. Introduction

Troponin T is part of the contractile apparatus that reversibly binds to tropomyosin in cardiac myofibrils. Troponin is the biomarker used to diagnose myocardial infarction [1]. Highly sensitive methods for measurement of troponin are now available in many hospital laboratories. Unfortunately, currently available point-of-care methods are not able to achieve the level of analytical sensitivity seen by laboratory-based instruments. One recent definition of a highly sensitive troponin measurement included a total coefficient of variation (CV) at the 99th percentile of 10%, and an ability to measure troponin below the 99th percentile at a concentration above the assay's limit of detection for at least 50%, and ideally 95%, of healthy individuals [2,3]. Devices that approach these limits of sensitivity are able to diagnose myocardial infarction with greater accuracy, and can identify individuals at high risk for future cardiovascular events [4–7].

The Elecsys 4th generation troponin T assay (Roche Diagnostics) is the only commercially available instrument used to measure troponin T in the U.S. It has a reported limit of detection (LOD) of 10 ng/l, a functional sensitivity (10% CV) of 30 ng/l, and is a laboratory-based chemiluminescent immunoassay. The Nanomonitor is a novel point-of-care device that measures the change in impedance that occurs when an antigen binds to an antibody, and is comprised of a polymeric nylon membrane overlaid on top of a gold microelectrode on a printed circuit board chip, which results in a high density array of nanoscale confined spaces. The Nanomonitor turns the impedance signal into a concentration for the target biomarker using electrochemical impedance spectroscopy. Prior work by our group showed that leveraging the phenomenon of macromolecular crowding can be utilized to design ultra-sensitive protein biosensors [8–13]. Macromolecular crowding theory postulates that the crowded environment in cells enhances protein interactions that are significantly different compared with protein interactions occurring in buffered solutions used in most laboratory-based measurement of proteins. The spatial confinement due to macromolecular crowding may significantly affect the behavior of proteins in solution, such as their stability and configuration [14].

Abbreviations: EIS, Electrochemical impedance spectroscopy; BSA, Bovine Serum Albumin.

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The efficient synthesis of proteins in vivo is dependent on a number of different molecular chaperones [15]. The Nanomonitor can tailor the size of the well to the protein of interest, serving as a molecular chaperone and thus maximizing this macromolecular crowding advantage.

2. Materials and methods

2.1. Collection of specimens

Heparinized plasma was collected from discard blood from forty patients presenting to Oregon Health & Science University Hospital with suspected myocardial infarction between September 2013 and February 2014, and frozen within 2 h of collection, Supplemental Table 1. Specimens were deidentified and maintained at −80 °C until analysis. The Oregon Health & Science University Institutional Review Board approved this study. Samples did not undergo >2 freeze/thaw cycles prior to measurement using the Elecsys or Nanomonitor. These forty patients had blood samples analyzed on both the Elecsys and the Nanomonitor.

2.2. Nanomonitor

Measurements made with the Nanomonitor are based upon changes in impedance that occur at the electrical double layer when an antigen binds to its antibody, and have been described in detail previously [8–13]. Briefly, a nanoporous nylon membrane is integrated onto a platform composed of a gold modified branched concentric circle electrode design. This results in the generation of a high-density array of nanowells for nanoscale confinement of proteins into size matched spaces — here the nanowells have a diameter of 200 nm and a depth of 250 nm. The base platform is connected to the nano-membrane using a biocompatible polymer manifold constructed from polydimethylsiloxane, and hermetically attached with a sealant. The manifold accomplishes microfluidic flow of the analyte over the Nanomonitor. Dithiobis succinimidyl propionate is used as the chemical linker to attach the biomarker antibody of interest to the gold electrode. We used troponin T antibody (US Biological). This antibody recognizes an epitope between amino acid 60 and 70, which is different than the Roche 4th and 5th generation troponin T assays which both use epitopes between amino acid 125–131 and 135–147 for detection and reporting antibodies, respectively.

Electrochemical impedance spectroscopy (EIS) quantifies and records the changes in impedance. Therefore, by characterizing the electrical double-layer change in impedance, an accurate estimate of the concentration of the target biomarker is achieved. This change in impedance is summed across approximately 2 million nanowells on the 20×20 mm^2 surface, and results are available in less than 15 min. Two solder connectors run from the Nanomonitor working and counter electrodes to an EIS device that then connects to a laptop computer. A software package receives the input from the EIS device and displays the calibrated output results.

One level of troponin T calibrator at 72 ng/l was used to calibrate the Nanomonitor, (Troponin T STAT CalSet, Roche Diagnostics). A total of 9 different operators, trained to use the Nanomonitor, performed all measurements with the device.

2.3. Measurement method

To measure the impedance, a sinusoidal voltage signal of 10 mV was applied to the electrodes using Gamry Reference 600 potentiostat with a frequency sweep between 50 and 1200 Hz. The prepared Nanomonitor was first treated with phosphate buffered saline (PBS, Thermo Scientific) and the impedance was measured. The gold microelectrodes were then functionalized with a thiol cross-linker molecule, DSP (Dithiobis succinimidyl propionate) dissolved in DMSO (Dimethyl sulfoxide). DSP (Pierce Biotechnologies) contains a disulfide bond that forms a strong covalent bond with the gold surfaces of the Nanomonitor. The Nanomonitor was then incubated with saturating concentration, 100 ng/l of anti-troponin-T antibody for 15 min, which is the concentration corresponding to the impedance saturation point. DSP has amine reactive NHS (N-hydroxysuccinimide) ester group at the end of its 12 Å spacer arm that binds to the primary amine group in the antibody molecule. Following antibody immobilization, the sensor was inoculated with SuperBlock (Thermo Scientific) to eliminate the non-specific protein interactions. In between each step, the sensor was washed off thrice with PBS to remove any unbound molecules. The baseline impedance was determined by taking impedance measurement at the PBS wash step after SuperBlock. Following the zero dose, the Nanomonitor was incubated with the sample collected from the patients and the impedance change was determined. The sample volume used at each step was at 100 μL.

An example of a dose-response curve is Fig. 1. Purified troponin T was diluted in PBS in a logarithmic fashion producing concentrations of 0.001, 0.01, 0.1, 1, 10, 100, and 1000 ng/l. The change in impedance increased with increasing concentrations of troponin T. The data points are the average of three replicates. To measure impedance, the same methods were used as described in the preceding paragraph. The impedance decreased with increasing concentration of antigen molecules, with a change in impedance between 20 and 90 Ω. The dose response shown is from the average of 3 replicates. The sensor response in Fig. 1 is represented as a percentage change in impedance between baseline impedance and impedance at antigen doses. The noise of the system was determined as impedance change between the zero dose step and Superblock step, which was 9 Ωs. The change in impedance decreased at 100 ng/l due to the saturation of available antibody sites for antigen binding.

2.4. Limit of blank/limit of detection

Analytical sensitivity of the Nanomonitor troponin T assay was assessed using zero and low concentration (0.625 ng/l) diluted troponin T Quality Control material, lot number 23573 (Bio Rad). The troponin T was diluted in 7% Bovine Serum Albumin (BSA). BSA was used to avoid the possibility of measurable troponin T present in human serum. Each concentration was measured four times. The limit of the blank (LOB) was defined as the mean of the blank + 1.645* SD of the blank. The limit of detection was defined as the mean of the LOB + 1.645* SD of a known low concentration sample [16]. The known low concentration sample, 0.625 ng/l, had a coefficient of variation (CV) of 9%.

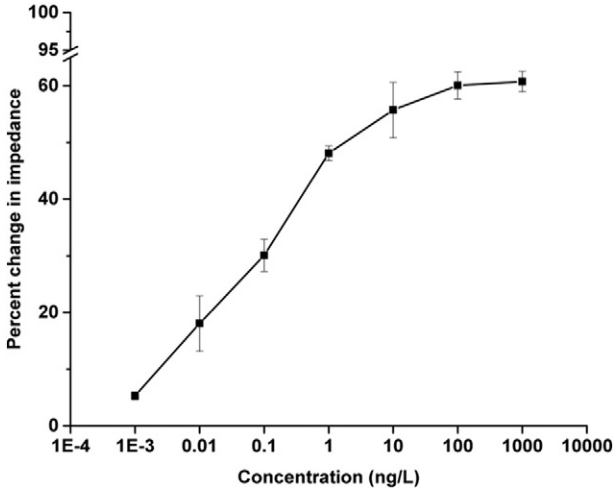

Fig. 1. Dose response curve. Troponin T in phosphate buffered saline.

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