EMS706U – Clinical Sensors and Measurements

Sensor Report

December 2025

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Clinical Relevance

Troponin is a protein which consists of three components – troponin I, troponin C and troponin T – that is involved, alongside actin and tropomyosin, in the contraction of muscle tissue (Filatov *et al.*, 1999). Troponin I (TnI) inhibits the actomyosin complex, which in turn, prevents a muscle contraction occurring. Troponin C (TnC) is the calcium ion (Ca^{2+}) binding subunit of troponin, and makes the actomyosin complex Ca^{2+} dependent, as well as removing the inhibition of TnI. Finally, troponin T (TnT) binds to tropomyosin as part of the thin filament structure of muscle fibres. The interactions between the troponin components, tropomyosin and the actomyosin complex are essential for the coordination of a muscle contraction response (Farah and Reinach, 1995).

Although troponin is found in skeletal muscle in various regions of the body, its clinical relevance is particularly seen through its presence in cardiac muscle. The previous gold standard biomarker for detecting myocardial damage and estimating the likelihood of ischemic heart disease was creatine kinase-MB. However, cardiac troponin assays are favoured because of their high specificity and sensitivity, which significantly influences early diagnosis of myocardial infarction (MI) in patients (Uettwiller-Geiger *et al.*, 2002). Troponin T concentration is suitable to measure because it has a short half-life of 90 minutes, and if there are continued elevated levels of TnT, this indicates degradation of myofilaments, hence causing irreversible cell damage (Katus *et al.*, 1991). TnT can, therefore, be used in cases with minor myocardial injury and acute coronary syndrome as a diagnostic method for MI. Additionally, the peak levels of troponin could provide details on the size of infarction (Al-Otaiby, Al-Amri and Al-Moghairi, 2011).

Using troponin as a key biomarker for myocardial injury can also give insights into the causes for increases in concentration of troponin and whether they can predict morbidity or mortality. For example, a review published by Aengevaeren *et al.* in 2021 explored whether exercise-induced cardiac troponin (cTn) elevations were clinically relevant, due to being the only benign form of troponin release. The study found that the level of post-exercise troponin release is attributed to both training status and exercise intensity, as higher-trained individuals require a greater absolute stimulus to elicit the same effect as a person who has lower training experience. Overall, the literature shows that measurements of cTn levels taken at rest are proficient predictors of cardiovascular morbidity and mortality, whereas exercise-induced increases are rarely used in practice for prognoses. Another study performed by Palazzuoli *et al.* in 2013 investigated the correlation between heart failure and renal impairment, classified as cardiorenal syndrome (CRS). This also represented the importance of cTn as an essential factor in prognosis of cardiovascular disease and how it can be translated to examine renal dysfunction.

Regular concentrations of cTn are very low and borderline undetectable, which greatly limits the transducers available for use in this application. The threshold is considered to be the 99^{th} percentile of a control reference group, as described in Al-Otaiby's paper, where cases below the 99^{th} percentile are characteristic of a healthy individual, and anything above is associated with MI. The specific values for concentration change with age and sex, but an analysis was conducted on three elderly patients – a 48 year-old man, a 60 year-old woman and a 54 year-old man – with underlying symptoms of MI, and the reported values of cardiac troponin I (cTnI) levels were found to be 0.05ng/ml, 0.06ng/ml and 0.06ng/ml, respectively, which were above the standard diagnostic limit of 0.04ng/ml (Mahajan and Jarolim, 2011). Despite the low sample size, this sheds light on how these low concentrations must be detectable using a specific type of transducer in the measurement of cTn. The transducers mentioned in this paper specifically describe the determination of cTnI as opposed to the other types of troponin, because this type facilitates early detection of acute myocardial infarction (AMI) and is widely used in most sensor-based technologies for heart-related diagnoses.

Transducer Technologies

The primary advantage of using sensors over traditional methods such as enzyme-linked immunosorbent assays (ELISAs) for the detection of cTn is the significant decrease in diagnosis time. By prioritising point-of-care (POC) testing, it has shown to reduce testing time from over 60 minutes down to 20 minutes (Docherty *et al.*, 2024). Other benefits include lower cost, higher sensitivity, and more interpretable results. Currently, the literature shows that the most effective transducer technologies for cTn determination are electrochemical immunosensors, including amperometric types, and impedimetric sensors.

Amperometric Sensors

An amperometric sensor is one which the analyte concentration is proportional to the measured current. A study performed by Docherty *et al.* in 2024 explored the effectiveness of a low-cost amperometric immunosensor for detection of troponin as a POC method for diagnosing acute myocardial infarction (AMI). Two versions of this sensor were created: a physisorption sensor and a chemisorption sensor. The capture antibody in the physisorption sensor was simply adsorbed onto the gold, whereas it was chemically attached using sulfo-LC-SPDP as a crosslinker in the chemisorption sensor. The sensor system consisted of a 96-well microplate reader, a thin gold film electrode with eight electrode channels, gold-sputtered polyester as the sensor substrate and finally a reference sensor printed using Ag/AgCl paste. The electrochemical results were recorded using a potentiostat, an MUX8 multiplexer for the eight electrodes, with measurements taken in chronoamperometry mode at -0.2V. A labelled schematic showing the experimental setup can be seen below in Figure 1.

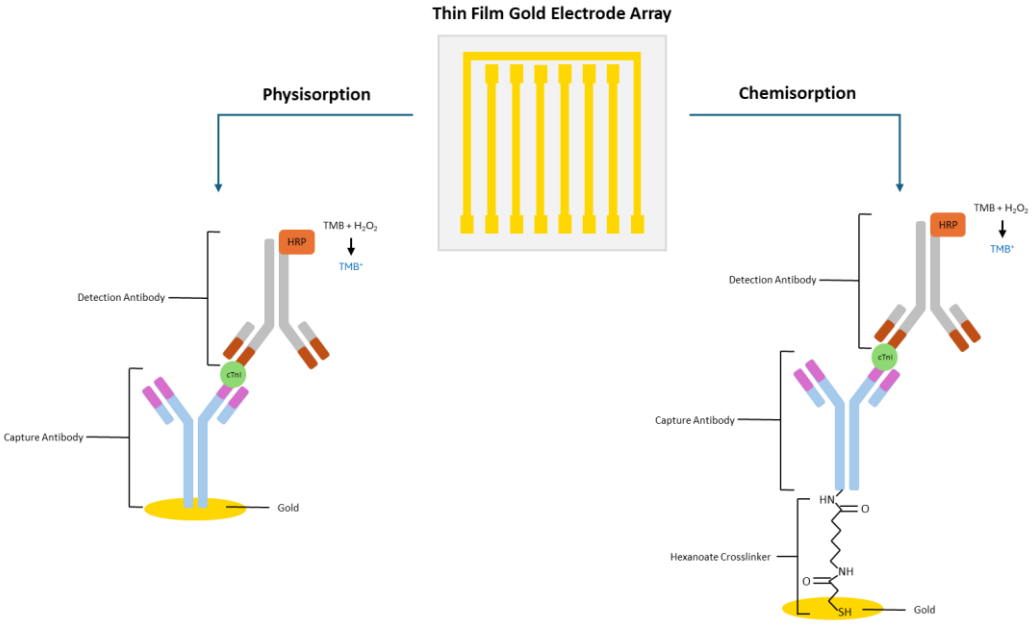

Figure 1 – Labelled schematic showing the physisorption and chemisorption sensor setup.

This sensor is adapted from the ELISA protocol and takes electrochemical measurements. Cardiac troponin I (cTnI) binds to the capture antibody immobilised on the gold electrode, with the addition of a detection antibody and horseradish peroxidase (HRP) enzyme, forming a sandwich. HRP reacts with 3,3′,5,5′-Tetramethylbenzidine (TMB), producing TMB^+ which is electroactive. A current can then be measured when the electrode is at -0.2V, as TMB^+ is reduced. A higher troponin concentration is associated with an increased current as a result of higher TMB^+ concentration.

The benchmark for this experiment was based on commercial ELISA measurements, which was found to be a limit of detection (LOD) of 159pg/ml in buffer solution and 84.5pg/ml in 10% human serum. The physisorption

sensor had a LOD of 309pg/ml and 233pg/ml in buffer and 10% human serum, respectively, whereas the chemisorption sensor had a much lower LOD of 109pg/ml in buffer and 130pg/ml in human serum, suggesting that using a crosslinker to chemically attach the capture antibody resulted in improved detection compared to the benchmark, and therefore provided the most accurate results for troponin concentration in this study.

Another system, created by Jo *et al.* in 2017, involved an aptamer-based amperometric sensor built on a screen-printed carbon electrode (SPCE). The key features of this sensor included a carbon working electrode, a reference electrode made from Ag/AgCl, and a carbon auxiliary (counter) electrode. The working electrode surface was modified by dropping gold nanoparticles and electrodepositing using cyclic voltammetry (CV). TTCA polymer was then electropolymerised via CV, and finally, Tro4 aptamer was immobilised onto the surface of the electrode to allow binding of the target cTnI.

Similar to the previous sensor, a sandwich structure is formed, instead using hydrazine-modified Tro6 aptamer bound to troponin, as opposed to HRP. The amperometric signals are generated through a reduction reaction of H_2O_2, with hydrazine acting as an electrocatalyst. As a result, when H_2O_2 is added and a constant voltage of -600mV is applied, the rate of electron transfer determines the current produced. Thus, a higher rate of electron transfer corresponds to a stronger current, which in turn, suggests an increased troponin concentration.

The results of this experiment showed that this sensor had a LOD of 24pg/ml, reportedly in both a buffer solution as well as human serum, demonstrating outstanding detection relative to the existing cutoff levels for cTnI, which were 70pg/ml for the 99th percentile and 400pg/ml for the clinical cutoff values. Also, it is noted that the dynamic range for the sensor was relatively wide, between 0.024ng/ml and 2.4ng/ml, indicating a high sensitivity over a broader span of troponin concentrations. Overall, this sensor performed at a significantly high accuracy compared to other commercially available POC equipment such as Cardiac Triple, Troponin I Rapid Card and Elecsys Troponin I assay, as mentioned in the paper.

Impedimetric Sensors

Impedimetric sensors focus on measuring changes in impedance at the interface of a biorecognition surface. They are generally label-free, providing advantages including reduced cost, simpler design and the ability to measure a target's concentration without affecting the environment by adding a dye or tag (Nemati *et al.*, 2022).

In 2019, Shreyas Vasantham *et al.* proposed a POC impedimetric immunosensor for the detection of cTnI. The sensor was fabricated on a cellulose paper substrate using SPCEs to create a microfluidic paper-based analytic device (µPAD). Using paraffin wax, a microfluidic well decorated the top of the electrode, functioning as an electrochemical cell. Multi-walled carbon nanotubes (MWCNTs) were drop casted onto the working area to prepare the bare paper electrode. 50ng/ml of anti-cTnI (antibody) was immobilised on the surface of functionalised MWCNT and incubated to facilitate interaction. Electrochemical impedance measurements were performed in phosphate-buffered saline (PBS), with 5mM $[Fe(CN)_6]^{3-/4-}$ surface-attached redox probe, under optimised conditions. Human serum samples were prepared by spiking each with fixed volumes (~2 µl) of varying cTnI concentrations. The diagram in Figure 2 below illustrates each component of the sensor system.

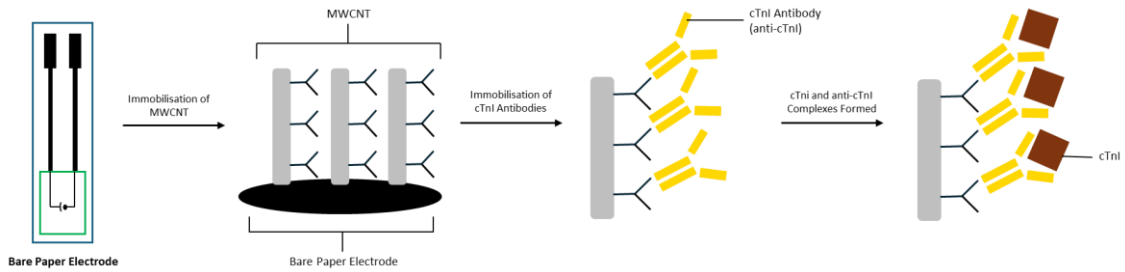

Figure 2 – Diagram showing sensor setup and cTnI binding to antibodies on MWCNTs.

When cTnI binds to the anti-cTnI present on the MWCNT surface, the complex acts as a kinetic barrier for electron transfer between the electrode and redox probe at the interface. This blockage is known as charge-transfer resistance (R_{ct}), which increases as more antigen-antibody complexes are formed. As a result, the impedance of the system is increased, given that frequency and surface properties remain constant. Electrochemical Impedance Microscopy (EIS) is used as a method of monitoring and analysing changes in impedance at the immunosensor interface. In this experiment, the frequency ranged from 100 Hz to 100 MHz.

With regards to the results of this sensor, the R_{ct} was measured at three occasions; the first was MWCNT alone, the second was with the addition of anti-cTnI, and the final measurement was made after antigen-antibody complexes were formed. The R_{ct} for each was found to be 50kΩ, 80kΩ and 140kΩ, respectively, confirming that the rate of electron transfer is reduced as more troponin binds to the antibodies on the immunosensor. The LOD of the sensor was recorded as 0.05ng/ml at a concentration range of 0.05-90ng/ml, which is exceptional relative to traditional ELISAs and radioimmunoassay (RIA) measurements. The sensitivity of the immunosensor was 1.85mΩ/ng/ml, with a response time of approximately one minute, emphasising the speed of POC sensors. A graph of R_{ct} against cTnI concentration was plotted, which showed a positive correlation between the two variables, backed up by a R^2 value of 0.97. Finally, the specificity of the sensor was tested by using a non-specific antigen (myoglobin), which did not produce a significant response, suggesting that the sensor had a high selectivity for troponin. Consequently, this sensor proved to be successful when compared to other POC sensors highlighted in the study, including amperometric and potentiometric models.

Similar to the second amperometric sensor mentioned earlier, an aptamer-based sensor for impedimetric cTnI detection was presented by Kitte, Bushira and Soreta, in 2021. It was designed using a standard electrochemical cell with three electrodes – ITO/AuNP/Tro6, platinum wire and Ag/AgCl as the working, auxiliary and reference electrodes, respectively. Thiol-modified Tro6 aptamer was immobilised onto the surface of AuNPs, hence becoming a part of the working electrode. The last key component was a redox probe consisting of PBS and 5 mM $[Fe(CN)_6]^{3-/4-}$ solution. All measurements were carried out via EIS, to analyse changes in impedance, and cyclic voltammetry (CV), to verify if the surface had successfully been modified at each fabrication stage. The sensor was tested in human serum and human urine for applicability to clinical settings.

Much like the previous impedimetric sensor, the charge-transfer resistance (R_{ct}), which is dependent on the extent of electron transfer blockage on the surface, is the main factor causing a change in impedance. The redox probe and working electrode exchange electrons, and the rate of electron transfer is reduced by surface modification, including aptamer immobilisation and binding of cardiac troponin onto Tro6, thus increasing R_{ct}. Therefore, by measuring the initial R_{ct} before cTnI is added, the change in resistance (ΔR_{ct}) can be calculated at various troponin concentrations (C_{cTnI}), and a relationship between ΔR_{ct} and $\log(C_{cTnI})$ can be plotted.

This sensor performed excellently with regards to sensitivity, specificity and reproducibility. It had a LOD of 0.055pg/ml recorded at troponin concentrations ranging from 0.1pg/ml to 10ng/ml, demonstrating remarkable detection ability. The selectivity of the Tro6 aptamer was tested using possible interfering species, including thrombin, myoglobin and other substances, and these did not elicit an impedance signal from the sensor, indicating its high specificity towards cardiac troponin. The calibration equation for ΔR_{ct} and $\log(C_{cTnI})$ produced a R^2 value of 0.992, implying strong linearity and positive correlation between the two variables. This experiment also recorded a relative standard deviation (RSD) of ~5%, highlighting the high degree of reproducibility. Overall, this aptamer-based model displayed outstanding results in determining cardiac troponin levels in human serum and urine relative to other impedimetric sensors, such as GCE-based, MWCNT/μPADs, and Tro4 aptamer-based sensors, as shown in the article.

Discussion and Conclusions

Both of the amperometric sensors were very successful in detecting low concentrations of cardiac troponin, down to the picogram (pg) level. However, based on the LOD of each sensor, it can be seen that the aptamer-based model was the most accurate and performed at a lower detection limit. The lowest concentration of troponin detected by immobilising Tro4 aptamer was 24pg/ml in buffer and human serum, which is substantially lower than the best-performing chemisorption sensor in the study with Docherty *et al.*, which had a reported LOD of 109pg/ml and 130pg/ml in buffer and 10% human serum, respectively. Assuming the same level of noise in both experiments, this suggests that the aptamer-based sensor could detect a lower level of troponin to a higher accuracy. The dynamic range for the electrochemical ELISA sensor was not explicitly stated, whereas the aptamer-based sensor could provide a trusted result for troponin concentration over a suitable range of 0.024-2.4ng/ml. Given this information, it should be noted that the electrochemical ELISA model prioritised affordability and commercial, off-the-shelf fabrication, meaning that a certain degree of sensitivity and/or accuracy may have been sacrificed. Overall, both sensors performed exceptionally well for the purpose of measuring cardiac troponin, highlighting the effectiveness of amperometry in this application.

Comparing the results from both impedimetric sensors, the aptamer-based sensor could detect a significantly lower concentration of cardiac troponin than the µPAD, as evidenced by a LOD of 0.055pg/ml as opposed to 0.05ng/ml. However, the µPAD was only tested in a concentration range of 0.05-90ng/ml, and it was able to detect the lower limit of this range, meaning there could be potential for a lower recorded LOD if tested using lower levels of troponin than in the study. Additionally, the reported LOD for the aptamer-based sensor was derived from a linear regression model. Although the data showed a reliable fit ($R^2 = 0.992$), the LOD was not tested under practical conditions below the concentration range, which may have influenced the final outcome. Also, the µPAD prioritised point-of-care (POC), response time and reduced cost over analytical performance, likely compromising on accuracy and sensitivity as a result. Both sensors showed a linear, positive correlation between charge-transfer resistance (R_{ct}) and troponin concentration, and produced high R^2 values (0.97 and 0.992 for the µPAD and aptamer sensors, respectively), indicating that each sensor strongly fit the linear regression models. A high specificity was also seen in both with the addition of non-specific interfering species, meaning that the sensors could correctly identify troponin in the presence of other potential interferences. Some miscellaneous results from each experiment included a response time of ~1 min for the µPAD sensor and a sensitivity of 1.85mΩ/ng/ml, and for the aptamer-based sensor, a RSD of ~5%, proving both sensors to be highly responsive and sensitive. With regards to the objectives of each sensor, both were very successful in achieving their respective goals and measuring cardiac troponin levels at a clinical standard, supporting the use of impedimetric sensors in this application.

In conclusion, both amperometric and impedimetric transducer technologies performed excellently in the detection of cardiac troponin concentration. Among those that highly prioritised POC testing (specifically the antibody-based sensors), the results show that a high degree of accuracy and sensitivity were displayed, despite the potential compromises made to performance. All sensors outlined in this paper exhibited excellent limit of detection (LOD), particularly the amperometric aptamer-based sensor and the two impedimetric sensors. With reference to the diagnostic limit of 0.04ng/ml published by Mahajan and Jarolim, the amperometric electrochemical ELISA model could not reach this level of detection. However, this may be insignificant as the sensors could likely detect an elevated level of cTnI, meaning it could diagnose acute myocardial infarction (AMI), as intended. Furthermore, each sensor from both transducer types closely mimicked the theoretical relationships between cardiac troponin concentration and their respective variables (current for amperometric measurements and R_{ct} for impedimetric measurements). Therefore, the literature clearly shows that both sensor types are viable options for this application.

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