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Diagnostics on acute myocardial infarction: Cardiac troponin biomarkers

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ABSTRACT

Acute myocardial infarction or myocardial infarction (MI) is a major health problem, due to diminished flow of blood to the heart, leads to higher rates of mortality and morbidity. Data from World Health Organization (WHO) accounted 30% of global death annually and expected more than 23 million die annually by 2030. This fatal effects trigger the need of appropriate biomarkers for early diagnosis, thus countermeasure can be taken. At the moment, the most specific markers for cardiac injury are cardiac troponin I (cTnI) and cardiac troponin T (cTnT) which have been considered as 'gold standard'. Due to higher specificity, determination of the level of cardiac troponins became a predominant indicator for MI. Several ways of diagnostics have been formulated, which include enzyme-linked immunosorbent assay, chemiluminescent, fluoro-immunoassays, electrical detections, surface plasmon resonance, and colorimetric protein assay. This review represents and elucidates the strategies, methods and detection levels involved in these diagnostics on cardiac superior biomarkers. The advancement, sensitivity, and limitations of each method are also discussed. In addition, it concludes with a discussion on the point-of care (POC) assay for a fast, accurate and ability of handling small sample measurement of cardiac biomarker.

Contents

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1. Introduction

James Bryan Herrick, an American physician was among the first to describe the symptoms of myocardial infarction (MI) (James, 2000). Herrick (1912) suggested, the symptoms and abnormalities of heart attacks was led by thrombosis in the coronary artery and this was not inevitable fatal. Thrombosis is obstruction of the blood flow through the circulatory system due to the formation of a blood clot inside a blood vessel. According to the pathology, MI is defined as myocardial necrosis (cell death) due to prolonged ischemia, reduction of blood supply to the heart (Thygesen et al., 2007). It is considered as the main cause of death and disability globally, and estimated 17.3 million people died in 2008, which over 80% of death take place in low- and middle-income countries. Moreover, by 2030, it is expected 23.3 million will die annually from cardiovascular disease (WHO, 2014).

Electrocardiograms (ECG) are a current method to measure and diagnose abnormal rhythms of the heart and helps to diagnose damage to the conductive tissue that carries electrical signals. However, ECG lacks sensitivity, although still remained as the recommended test to identify patients with MI (Zhang and Ning, 2012). The primary limitation of ECG is that only electrocardiographic activity at a single moment in time is represented; thus it usually needs to be done multiple times as a patient's clinical condition changes (Leisy et al., 2013). The second limitation is a subjective interpretation in the final analysis by the reading physician even though wave-pattern recognition and comparison with expected normal findings are used in ECG assessment. Thirdly, ECG is not useful for patients with non-ST segment (the contraction waves segment in the ECG representation) elevation myocardial infarction (NSTEMI), and found normal (Mahajan and Jarolim, 2011). Finally, an ECG is useful in identifying the presence of acute myocardial ischemia, a history of myocardial infarction, or the presence of a conduction defect or arrhythmia, but it is a highly unreliable test for establishing the presence of early coronary artery obstruction. To overcome these limitations and issues with ECG, the alternate strategy is the usage of potential cardiac biomarkers, which would be applicable for sensing purposes.

2. Cardiac biomarkers

Cardiac biomarkers are the indicators, which have been predominantly used in the detection of MI. The earliest documented study of MI based on biomarker has begun since 1954 (Dewar et al., 1958; Ladue and Wroblewski, 1955) focusing on glutamate oxaloacetic transaminase. It is logical to use protein quantification in a blood sample for this purpose as stated by Rosalki et al. (2004), i.e. the myocyte is the major cell in the heart, and the heart's purpose is to pump blood. When myocytes essentially cannot be regenerated due to heart cells die, then cardiac function has a high probability of being damaged. When the cell dies, the biomarker proteins (i.e. myoglobin, creatine-kinase MB, C-reactive protein and cardiac troponin are most commonly used) inside the cell will be released, with proteins in the cytoplasm leaving the cell more rapidly than the ones in membranes or fixed cell elements.

For MI, cardiac troponin T (cTnT) and cardiac troponin I (cTnI) are regarded as more sensitive and specific than other cardiac biomarkers i.e, myoglobin and creatine-kinase MB (Jaffe and Ordonez-Llanos, 2010). Both are released from the death cell within 2–4 h and 3–4 h, respectively, after the onset of MI symptoms (Burcu Bahadır and Kemal Sezgintürk, 2015). Some results are favorable for cTnI (De Antonio et al., 2013), but the comparison was made between high sensitive cTnI with sensitive cTnT (Hetland and Dickstein, 1998). In principle, cTnT and cTnI remain in the blood stream approximately more than 10 days, reaches to peak approximately 1–2 days (Thygesen and Alpert, 2000) after myocardial injury. Because of its prolonged release in the blood, these biomarkers are useful diagnosing sub-acute myocardial infarction (Jaffe and Ordonez-Llanos, 2010). As cardiac troponin is cardiac-specific biomarker, it helped in isolating cardiac from skeletal muscle or other organs damage (McDonough and Van Eyk, 2004). In normal patients, the level of cTnI concentration is around 0.001 μg/L, but increased to 100 μg/L in MI patients (Agewall et al., 2011). Even the concentration as low as 0.01 μg/L can be related to heart failure. An increased value for cardiac troponin should be defined as a measurement exceeding the 99th percentile of a reference control group (Thygesen and Alpert, 2000). Reference values must be determined in each laboratory by studies using specific assays with appropriate quality control. Acceptable imprecision (coefficient of variation) at the 99th percentile for each assay should be defined as less than or equal to 10%. Fig. 1 shows general information regarding myocardial infarction.

In addition, there is another biomarker called troponin C (cTnC) (Takeda et al., 2003). It is originally from the 3-unit troponin complex (troponin I, T and C) along with tropomyosin, located on the actin filament. It is needed for the calcium-mediated regulation of skeletal and cardiac muscle concentration. Unfortunately, cTnC has no cardiac specificity due to the reason that cardiac isoform of troponin C is shared with slow-twitch skeletal muscles, which made it less favorable to be used as cardiac biomarker, unlike cTnI and cTnT for the diagnosis of cardiac injury.

3. Detection and quantification methods of cTnI and cTnT

In this case, biosensors can be used to detect and quantify the target molecules involved with cardiac biomarker interaction. Biosensors are integrated diagnostic devices, which merge biological or biologically-derived sensing element associated with a physicochemical transducer (Mascini and Tombelli, 2008). Generally, surface of a suitable transducer of a biosensor is immobilized with a biological receptor material (DNA, RNA or antibody), which enables conversion of biochemical signal into quantifiable electronic signal (Qureshi et al., 2012), through the mode of either electrochemical (Gomes-Filho et al., 2013; Horak et al., 2015), optical (He et al., 2013; Leung et al., 2013, 2015; Lu et al., 2014), mass change (piezoelectric/acoustic wave) (Lee et al., 2013), or magnetic (Liu et al., 2014). Compared to the conventional technique such as ECG, biosensors possess high sensitivity, high selectivity, fast analysis, reliable pretreatment and simple instrumentation (Burcu Bahadır and Kemal Sezgintürk, 2015). Different methods have been developed for cardiac troponin detection and quantification which include enzyme-linked immunosorbent assay (De Antonio et al., 2013), chemiluminescent immunoassay (Cho et al., 2009), fluoro-immunoassays (Hayes et al., 2009), electrical detections (Tuteja et al., 2014), surface

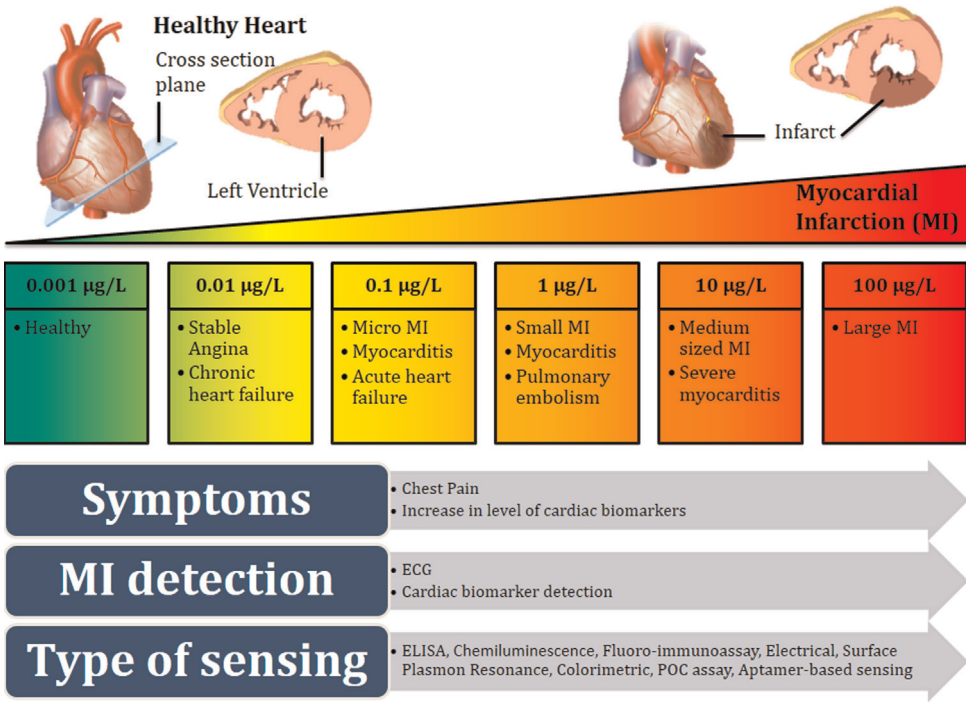

Fig. 1. Diagnostics on myocardial infarction (MI). Troponin concentration level, symptoms, current methods of MI detection, and methods of cardiac biomarker detection are described.

plasmon resonance (SPR)-based detection (Liu et al., 2011), colorimetric protein array (Wu et al., 2010), point-of-care (POC) assays (Dittmer et al., 2010), and aptamer-based biosensor (Shu-hai et al., 2014). This review focus on the advancement, sensitivity and limitations of these methods towards detection of cardiac troponin biomarkers. Table 1 displays the involvement of different diagnostics on MI using cardiac troponin as the biomarkers.

3.1. Enzyme-linked immunosorbent assay (ELISA)

ELISA is a biochemical assay that utilizes antibodies and an enzyme-mediated color change to identify the existence of either antigen (proteins, peptides, hormones, etc.) or antibody in a given sample (Gan and Patel, 2013). Detection of very small quantities of antigens is permitted by using fundamental concepts of immunology of an antigen binding to its specific antibody, and has been applied in the detection of cardiac troponin to diagnose MI. The first article on ELISA published by Engvall and Perlmann (1971) demonstrated quantitative measurement of IgG in rabbit serum using alkaline phosphatase as the reporter label. In ELISA, the antigen is allowed to bind to a specific antibody, which itself afterwards is detected by a secondary, enzyme-coupled antibody that reacted with a chromogen. The existence of antigen is indicated by the production of a visible color change or fluorescence from a chromogenic substrate for the enzyme. This color change can be quantitative or qualitatively measured to detect the antigen (Fig. 2).

The first generation of troponin T ELISA (TnT 1) was developed by Katus et al. (1992), which can be considered more sensitive enzyme immunoassay using two troponin T-specific monoclonal antibodies. The method was based on the one-step sandwich assay principle, the antigen was bound to the streptavidin-coated polystyrene tubes as the solid phase, by an affinity-purified polyclonal antibody from sheep and detected by peroxidase-labeled monoclonal antibody. The assay is carried out at room temperature for 90 min, measuring range is 0.1–15 µg/L. Despite that, this assay is unreliable in patient with severe skeletal muscle injury due to increased false-positive result (Muller-Bardorff et al., 1997) due to unspecific binding of skeletal muscle troponin T to the wall of the test tube, which can then be detected by the cross-reactive enzyme-labeled antibody used in the TnT 1 assay.

The second generation of cardiac-specific troponin T ELISA (TnT 2) was developed by Muller-Bardorff et al. (1997), in which the cross-reactive antibody 1B10 has been replaced by a high affinity cardiac specific antibody M11.7. The substantial improvement for this ELISA, in term of specificity, is the ability to differentiate between cardiac and skeletal muscle damage, even in patient with severe skeletal muscle injury. The detection limit is 0.012 µg/L with co-efficient of variation (CV) $<$ 5.8%. Reduction of turnaround time for 45 min without loss in analytical precision and clinical sensitivity has been achieved.

Next, Hallermayer et al. (1999) reported on the Elecsys® Troponin T third generation assay which used recombinant human cTnT as standard material, thus allows a reproducible and reliable standardization of troponin T assays. The assay has high precision, especially at the low end of measuring range (inter-assay CV $<$ 10% at 0.1 µg/L). The new standardization does not change the cutoff value of 0.1 µg/L. The new assay has a linear calibration curve; thus linearity problems observed with the second-generation assay have been eliminated.

As for cTnI, it was developed by Bodor et al. (1992), a double monoclonal sandwich enzyme immunoassay to measure cTnI in serum. The assay required $<$ 4 h to perform and used an enzyme label for detection with minimum detectable dose of 1.9 µg/L. The use of monoclonal antibodies allowed greater reproducibility of reagents than the polyclonal antiserum. The ELISA was quite suitable for evaluating the value of cTnl measurements, but was not rugged and precise enough for high-volume routine laboratory use.

Even though, ELISA has been used for the detection of cardiac troponin biomarkers, it has some hurdles as it suffers from important sample and reagent consumption in large scale studies; need to be performed in central laboratories of clinic and hospitals; and take a very long time to perform (more than 6 h), which

Table 1
List of cardiac troponin biomarker detection methods with their probe type, sensitivity, and advantages.

Method	Probe	Lowest detection limit of troponin (μg/L)	Advantages	Reference
Enzyme-linked immunosorbent assay				
1st generation (TnT 1)	MAb-TnT	0.1	Sensitive	Katus et al. (1992)
2nd generation (TnT 2)	Mouse MAb-cTnT	0.012	Cardiac specific	Muller-Bardorff et al. (1997)
3rd generation cTnT (Elecsys®)	MAb-cTnT	0.1	Eliminate linearity problem	Hallermayer et al. (1999)
Double monoclonal sandwich enzyme immunoassay cTnI	MAb-cTnI	1.9	Greater reproducibility of reagent	Bodor et al. (1992)
Chemiluminescence				
Chemiluminometric EOC	Anti-cTnI	0.027	Fast detection	Cho et al. (2009)
Screen-printed (SP) microarray	MAb-cTnI, MAb-Myo, MAb-CRP, MAb-BNP	0.06	Multiple cardiac biomarker detection, highly sensitive	Marquette et al. (2009)
Mitsubishi PATHFAST®	Mouse MAb-cTnI	0.003	Rapid, sensitive, quantitative	Kurihara et al. (2008)
Fluorescence immunoassay				
Fluoro-microbead guiding chip (FMGC)-based sandwich immunoassay	MAb-cTnI	0.1	Highly sensitive	Song et al. (2011)
Electrical detection				
CMOS-compatible SiNW array	Mouse MAb-cTnT	0.000001	Label free, ultrasensitive, real time detection	Chua et al. (2009)
Multiplexed detection CMOS-compatible SiNW array	Anti-cTnT, anti-CK-MM, anti-CK-MB	0.000001	Highly sensitive and selective, real time detection	Zhang et al. (2009b)
SiNW FETs CMOS	MAb-cTnI	0.092	High sensitivity and selectivity, mass production ability, anti-interference	Kong et al. (2012)
Single PANI nanowire	MAb-cTnI, MAb-Myo, MAb-CK-MB, MAb-CK-MM	0.00025	Ultra-high sensitivity, good sensing reproducibility, high specificity	Lee et al. (2012)
Functionalized SnO_2 nanobelt FETs with integrated microfluidics	Anti-cTnI	~2	Real time, label free, Portable	Cheng et al. (2011)
CNT supported by a conductive polymer film	MAb-cTnT	0.033	High sensitivity, good sensing reproducibility and repeatability	Gomes-Filho et al. (2013)
Amine functionalized CNTs, incorporated into the ink printing used to fabricate screen printed electrode (SPE)	Mouse MAb-cTnT	0.0035	Better stability in measurement, high sensitivity, label-free	Silva et al. (2013)
Monolithic graphene sheets	MAb-cTnI	~0.0001	Highly sensitive, label-free	Tuteja et al. (2014)
Surface plasmon resonance				
Optical-based-SPR	Mouse MAb-cTnI	0.25	Enhanced sensitivity	Wei et al. (2003)
Fiber-optic-based SPR sensor	Mouse MAb-cTnI	1.4	Fast	Masson et al. (2004)
Quick detection SPR	Mouse Biotinylated MAb-cTnT	0.01	Cardiospecific, Highly sensitive, Fast	Dutra and Kubota (2007)
SPR sensor on a commercially available SPR AUTOLAB SPIRIT®	Biotinylated MAb-cTnT	0.05	Specific, Good reproducibility	Dutra et al. (2007)
self-assembled monolayer (SAM) with high anti-fouling ability	Anti-cTnT	100	high detection capability, high accuracy and reproducibility, rapid, label-free	Liu et al. (2011)
Colorimetric detection				
Poly(dimethylsiloxane) (PDMS)-gold nanoparticles (AuNPs) composite film-based	MAb-cTnI	0.01	Fast detection, low cost	Wu et al. (2010)
Aptamer				
Aptamer-based biosensor	Aptamer	1.1938	Fast detection	Shu-hai et al. (2014)

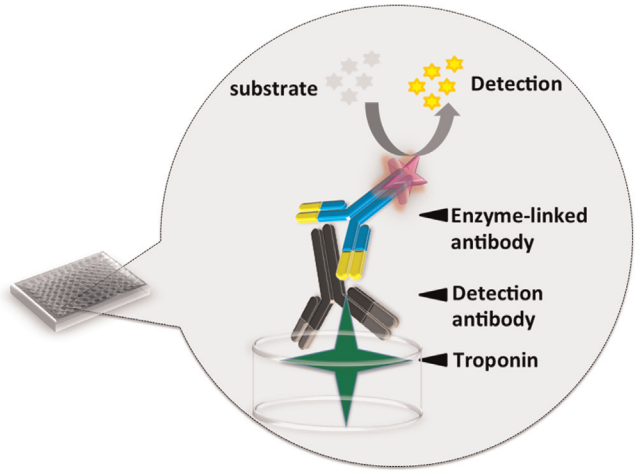

Fig. 2. ELISA used to detect antigen in a given sample. Different concentrations of the troponin can be titrated. Appropriate primary antibody to be used for binding. For detection, enzyme-conjugated anti-rabbit/mouse need to be used as a secondary antibody. Substrate is used for the color development and measured at an appropriate wavelength.

is incompatible with the quick decision needed to treat a heart attack patient (Zhang and Ning, 2012).

3.2. Chemiluminescence immunoassays

Chemiluminescence (CL) is a term used to represent light emission, happens whenever a molecule emits a photon in an excited state (energy is produced by chemical reaction) and relaxes to its ground state (Dodeigne et al., 2000). When CL systems are combined with the immunoreactions, they became a method to determine the concentrations of samples according to the intensity of the luminescence that the chemical reaction emit (Pei et al., 2013), called the chemiluminescence immunoassays (CLIA).

The system generated CL by the introduction of the CL substrates i.e., luminol, isoluminol, and their derivatives, acridinium ester, derivative, peroxidase and alkaline phosphatase (ALP) to some reagents which acts as CL labels (Wang et al., 2012). To label proteins in CLIA, the most commonly used labeling enzymes are horseradish peroxidase (HRP) and ALP. CL has been used as a label in immunoassay by Schroeder et al. (1976), who first described a method for monitoring competitive protein binding reactions by using CL.

Based on the method by Schroeder et al. (1976), then Cho et al. (2009) developed a chemiluminometric ELISA-on-chip (EOC) biosensor in combination with an image detector equipped with a cooled charge-coupled device (CCD) camera capable of detecting cTnI. The sensor used an immuno-chromatographic assay combined with an enzyme tracer that produces a light signal measureable on a simple detector. The cross-flow chromatography is utilized in order to accomplish sequential antigen–antibody binding and signal generation. Performing ELISA on a redesign plastic chip simplifies the fabrication process. Biotin–streptavidin capture technology was implemented to enhance the sensitivity in preparing an immuno-strip that was then merged onto the chip in order to create the EOC biosensor. By adding a luminogenic substrate to the tracer enzyme complexed with the analyte on the chip, a chemicaluminescent signal was generated proportional to the analyte concentration. A cooled charge-coupled device detected the luminescent signal in a dark chamber and the signal was translated to optical density for quantification. The biosensor systems were capable of cTnI detection present in serum at concentration 0.027 μg/L, 30 times lower than the conventional rapid test kit with colloidal gold as the tracer. It was faster (final result acquired 30 s after addition of the enzyme substrate) than the detection time required when using colorimetric substrate with the same tracer enzyme.

Marquette et al. (2009) have presented a screen-printed (SP) microarray, a platform for the achievement of multiparametric biochips. It is composed of eight (0.28 mm^2) working electrodes modified with electro-addressed protein A-aryl diazonium adducts. The electrode surfaces are then used as an affinity immobilization support for the orientated binding of capture monoclonal antibodies, having specificity against cTnI. The immobilized capture antibodies are involved in sandwich assays of the cTnI together with biotinylated detection antibodies and peroxidase-labeled streptavidin in order to permit a chemiluminescent imaging of the SP platform and a sensitive detection of the assayed proteins. The performances of the system in pure buffered solutions, using a 25-min assay duration, were characterized by dynamic ranges of 0.2–20 μg/L with limit of detection of 0.06 μg/L for cTnI. The assays were also validated in spiked 40-times-diluted human sera, using LowCross buffer, and were shown to work simultaneously in this complex medium.

A commercialized instrument based on CL immunoassay has also been developed by Kurihara et al. (2008), a CLIA known as Mitsubishi PATHFAST® to detect cTnI concentration. The system adopted highly sensitive chemiluminescent enzyme immunoassay (CLEIA) using CDP-Star/Sapphire-II (Applied Biosystems) as chemiluminescent substrate and Magtration (Obata et al., 2001) by which efficient bound/free (B/F) separation can be performed in a disposable pipette tip with a small volume. It has the quantification limit of cTnI of 0.007 μg/L, below the 99th percentile of the reference group (0.02 μg/L) and fulfilled the requirement of the guideline recommendation with imprecision (CV) at the 99th percentile of the reference group of $< 10\%$ (Fig. 3).

Researcher and clinical analysts have accepted

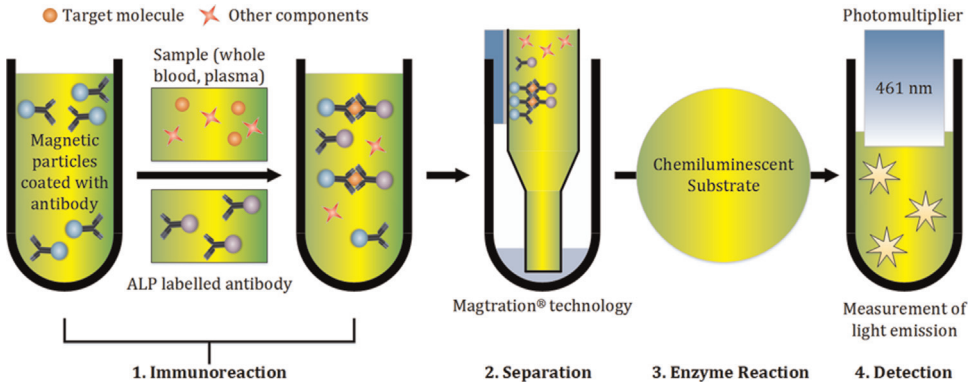

Fig. 3. Mitsubishi PATHFAST® principle of operations.

chemiluminescent immunoassay widely, which is enzymatically triggerable because of its high sensitivity, wide linear range, and easy operation and automation (Liu et al., 2014).

3.3. Fluorescence immunoassays

Fluorescence immunoassays (FI) are one of the optical biosensors' classification (Qureshi et al., 2012) involving the signal transduction of complexed molecules in homogeneous and heterogeneous assays. FI are preferable due to their non-destructive, highly sensitive characteristic and the ease of modifying the biomolecules with fluorescence tags. Fluorescence tags such as fluorescein isothiocyanate, rhodamine, coumarin, and cyanine are used as label or bio-recognition. The presence of the target molecules is indicated by the changes of the fluorescence signal. The detection limit is extremely sensitive, which is down to single molecule (Fan et al., 2008).

For FI, Song et al. (2011) developed a fluoro-microbead guiding chip (FMGC)-based sandwich immunoassay for cTnI detection. The FMGC offers the capability of using a fluorescence microscope to count the number of beads bound in the immunosensing region directly. Conjugation of antibody to fluoro-microbes is prepared as the detection component. The avidin–biotin affinity interaction was utilized to boost the antigen–antibody binding signal. Biotin-conjugated cTnI detection antibody was loaded into the chip after immobilization of the capture antibody, binding of the target antigen cTnI occurred and reacted for 30 min as shown in Fig. 4. After washing with PBS, 0.005% avidin-conjugated fluoromicrobeads were injected and counted directly by using a conventional fluorescence microscope. The optical signal showed a linear correlation with the cTnI concentrations in plasma samples containing 0.1–100 μg/L cTnI.

In spite of being highly sensitive, FI detection is bulky, expensive and required trained personnel to perform the tests (Qureshi et al., 2012).

3.4. Electrical detections

To overcome the limitations of immunoassay-labeled method such as, lack of portability, late detection time, and high complexity of fabrication process (Kong et al., 2012), the electrical detection of bio-molecular interaction development is highly beneficial because it is suitable to become the low cost portable sensor and can be used non-specialized personnel (Estrela et al., 2009).

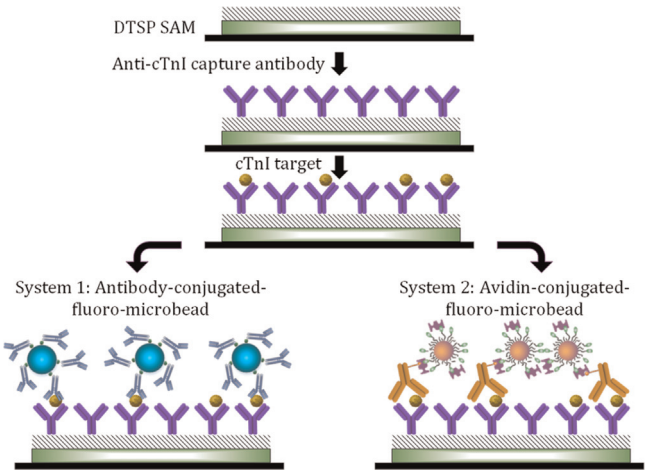

Fig. 4. Schematic diagram of the sandwich immunoassay using antigen/antibody binding (System 1) and avidin/biotin affinity binding (System 2) on the FMGC. The avidin/biotin couple was used to enhance the signal.

Electrical detection is conducted by transducing the molecular binding event into a usable electrical signal (Zhang and Ning, 2012). In order to sense chemical and biological species which is very small in size, researchers have intensely studied nanostructures, such as nanowires (NWs), nanobelts, carbon nanotubes (CNTs), graphene, and nanoparticles for biosensing, due to comparable size between sensor and target. The electrical detection has become the main interest in recent year, which was evidenced by higher number of publications related to diagnostic MI as discussed next.

3.4.1. Nanowire field-effect transistor

Chua et al. (2009) presented a CMOS-compatible Silicon Nanowire (SiNW) array platform for the label free, ultrasensitive, real time detection of cTnT in assay buffer as well as in undiluted serum sample based on top down method as shown in Fig. 5. It is achieved through electrical measurement based on the conductance changes of the individual SiNWs. Conventional Silane chemistry, 3-aminopropyltriethoxysilane (APTES) is used for the surface functionalization of the SiNW array chips to generate amine group (Zhang et al., 2009a); and glutaraldehyde, a bifunctional linker is used to immobilize antibody on the SiNW surface. Demonstration of human cTnT detection in assay buffer solution concentration and undiluted human serum environment had successfully detected down to 1 pg/L and 30 pg/L, respectively. This method has the advantage of eliminating the need for expensive, highly specialized equipment in diagnostic screening test.

The same year, Zhang et al. (2009b) developed a multiplexed detection of cardiac biormarkers (cTnT, CK-MM and CK-MB) based on the CMOS-compatible SiNW array platform for detection of cTnT in assay buffer as well as in undiluted serum sample. It is capable of detecting 1 pg/L cTnT in buffer and 30 pg/L in desalted serum, and allowed multiplexed detection of 100 pg/L of cTnT, creatine kinase-MM (CK-MM) and creatine kinase-MB (CK-MB) in untreated blood serum.

Kong et al. (2012) developed detection of cTnI using SiNW FETs CMOS compatible top-down method. Mab-cTnI was covalently immobilized on the SiNW surfaces. This device has a limit of detection down to 92 ng/L and has a linear dynamic range of 0.092–46 μg/L.

In recent study, Lee et al. (2012) had reported development of different material i.e., polyaniline (PANI) nanowire for detecting four cardiac biomarkers: cTnI, Myo, CK-MB, or BNP. PANI is organic material and more easily modified with biomolecules than inorganic nanomaterial (Tolani et al., 2009). The covalent bond between PANI and the antibody during surface functionalization of PANI allows direct measurement of physical change of conductance, capacitance, or impedance upon binding of antibodies to target proteins (Adhikari and Majumdar, 2004), other than controllable conductivity, mechanical flexibility and exceptional bioaffinity (Ahuja et al., 2007). The single PANI nanowire as shown in Fig. 6 was fabricated by electrochemical deposition growth method between pre-patterned Au electrodes, avoiding the need for the selection and alignment of nanowire. The single PANI nanowire was functionalized by covalently attaching the monoclonal antibody on the surface. The binding between immobilized MAbs and target biomarkers changes the net surface charge of the PANI nanowire and induces the carrier accumulation or depletion, which translates the changes in nanowire conductance. The detection as low as 250 pg/L, 100 ng/L, 150 pg/L, and 50 pg/L for cTnI, Myo, CK-MB, and BNP, can be achieved, respectively.

3.4.2. Nanobelt field-effect transistor

Possibility of using nanobelt field effect transistor was reported by Cheng et al. (2011), who showed detection of cTnI using functionalized Tin oxide (SnO_2) nanobelt field-effect transistors (FETs)

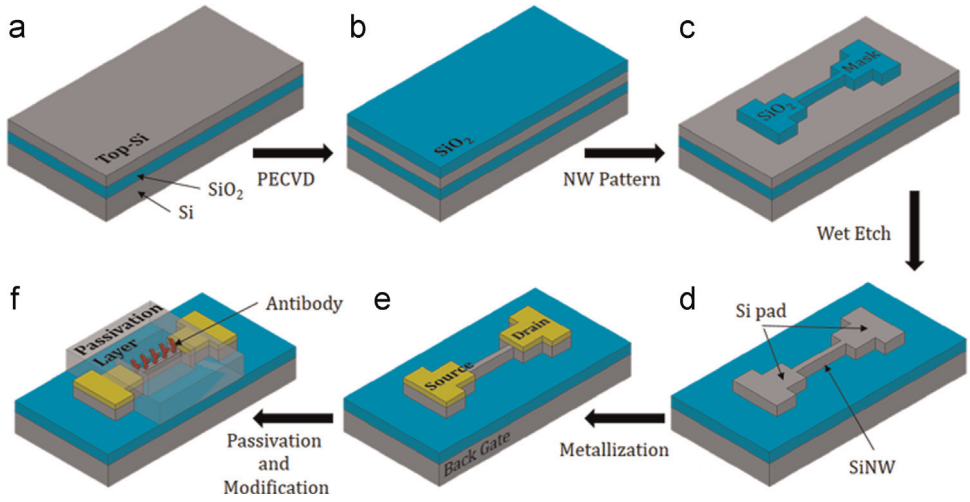

Fig. 5. Key workflow steps used for the fabrication of SiNW based FET devices. (A) Thermal oxidation to thin the device layer of the SOI wafer down to ~50 nm. (B) Thin film of SiO_2 deposited by PECVD. (C) Define SiO_2 pattern by lithography. (D) Crystallographic wet etching using the TMAH solution to obtain SiNWs. (E) Deposition of metal contact for source, drain and back gate electrodes, followed by rapid thermal annealing to acquire ohmic contact. (F) Fabrication of SiO_2/SiN*x* passivation layer by PECVD, lithography and RIE processes, followed by covalent modification of cTnI antibodies.

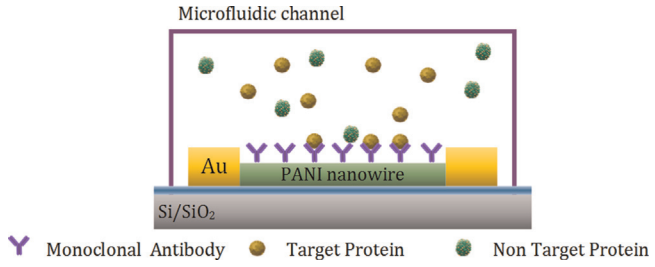

Fig. 6. The cross section view of the single PANI nanowire-based biosensor.

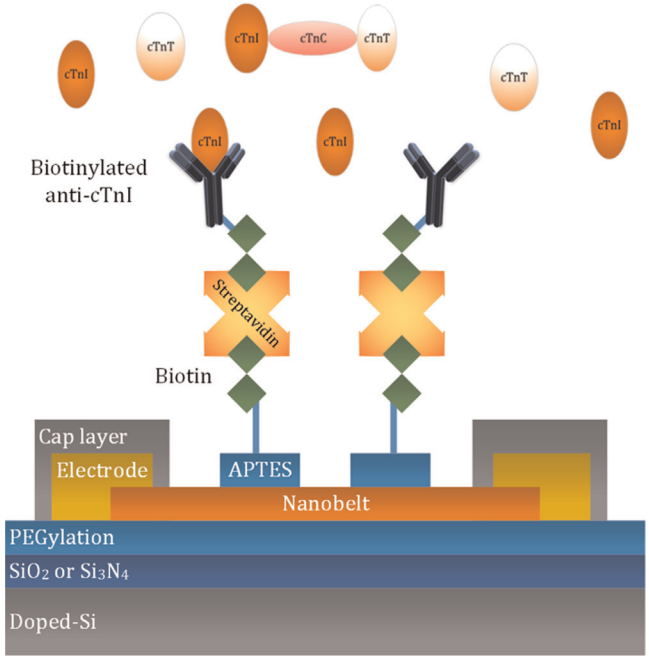

Fig. 7. Schematic diagram of the cTnI sensing scheme depicting the detailed assembly procedure of antibodies on the nanobelt surface and subsequent detection of the antigen.

with integrated microfluidics as shown in Fig. 7. Nanobelts of SnO_2, produced via catalyst-free physical vapor growth (Pan et al., 2001), are single crystalline and biocompatible, and their ribbon-like morphology maximizes surface-to-volume ratio. The nanobelt biotinylation is started with covalent APTES linkage on the oxide surface of SnO_2, followed by biotinylation by attachment of D-biotin, and substrate passivation by incubating in streptavidin solution for 6 h. Finally, the sensing platform is utilized for detection of cTnI with sensitivity of ~2 μg/L. The main advantage of this sensing scheme lies in its exceptional portability and detection speed.

3.4.3. *Carbon nanotubes (CNTs)*

The structure of the CNTs (a rolled-up tubular shell of graphite sheet with the carbon atoms covalently bound to their neighbors), their physical and chemical properties such as electrical conductance, high mechanical stiffness and the possibilities to functionalize CNTs in order to change their intrinsic properties (Yun et al., 2007) are the reasons being utilized. The CNT electrode can reach high sensitivity with low detection limits due to rapid electron transfer, thus increased reaction rate of many electroactive species and then decreasing the electrode response time (Janegitz et al., 2011; Laschi et al., 2008). In addition, CNTs increase the electroactive area by forming a nanostructured surface that promotes a greater amount of immobilized biomolecules (Gomes-Filho et al., 2013).

Due to their extraordinary properties, Gomes-Filho et al. (2013) had developed a nanostructured immunosensor based on CNT supported by a conductive polymer film for detection of cTnT. The electrode surface was covalently bound with carboxylated CNT via polyethyleneimine (PEI) which has a higher density of amine groups (Liu et al., 2010). In order to bind anti-cTnT monoclonal antibodies, the functionalized nanostructured surface was utilized. The immunosensor achieved a low limit of detection of 0.033 μg/L and a linear range between 1 and 10 μg/L cTnT, significant for acute myocardial infarction diagnosis. Good reproducibility and repeatability were obtained by the proposed immunosensor supported by a coefficient of variation of 3.7% and 2.6%, respectively.

In spite o the capabilities of cTnT detection down to 0.033 μg/L, the immunosensor developed by Gomes-Filho et al. (2013) can be related with instabilities in the response due to CNTs deposited and assembled on a polymeric film can leach during measurement process. To overcome this disadvantage, Silva et al. (2013) had developed an amine functionalized CNTs, incorporated into the ink printing used to fabricate screen printed electrode (SPE) to detect cTnT. A better stability in measurement is enabled by incorporating NH_2-CNT into the carbon ink. In addition, oriented

immobilization of anti-cTnT has led to a high sensitivity and very low detection level (0.0035 μg/L), almost 10 times lower than immunosensor reported by Gomes-Filho et al. (2013).

3.4.4. Graphene nanomaterial

Graphene, a monolayer of a hexagonal network of carbon atoms densely packed into a two-dimensional honeycomb crystal lattice, is characterized by fascination properties such as an ambipolar electric field effect along with ballistic conduction of charge, elasticity, superior thermal conductivity, and excellent mechanical, and electronic properties (Geim and Novoselov, 2007). Tuteja et al. (2014) had developed highly sensitive label-free electrochemical detection platform for cTnI by using monolithic graphene sheets from lithium ion intercalation mediated efficient exfoliation process. The carboxylated graphene (prepared by one step lithium ion intercalation mediated exfoliation process of graphite) is used as a gate-sensing material in between the source and drain gold inter-digitated electrode by dropcasting and incubation for 1 h at 120 °C. Carboxyl groups of functionalized graphene [activated by Carbodiimide hydrochloride (EDC) and N-hydroxysuccinimide (NHS)] were used for biointerface development using aqueous phase carbodiimide activation chemistry (Sharma et al., 2013). Next, the surface of the activated carboxylated graphene is immobilized with anti-cTnI antibody for cTnI detection. The detection limit of cTnI was achieved down to 0.0001 μg/L with linear response from 1 to 1 μg/L.

3.5. Surface plasmon resonance (SPR)

SPR is a charge–density oscillation that may exist at the interface of two media with dielectric constants of opposite signs, for instance, a metal and a dielectric (Homola et al., 1999). When the surface of a thin metal film is excited by an incident beam of light (with a suitable wavelength at a specific angle), this event generated an evanescent electromagnetic field and it is described as a charge density oscillation occurring at the interface between two media of oppositely charged dielectric constants. At the interface, the evanescent field generated under total internal reflection conditions is the strongest, but diminished as the distance of penetration from the surface increased. SPR supported the detection of only surface-confined molecular interactions occurring on the transducer surface (Dutra et al., 2007). Although there are two configurations used for excitation of surface plasmons, Kretschmann and Otto (Homola et al., 1999), Kretschmann configuration working at attenuated total reflectance for excitation of surface plasmons is widely used (Dutra and Kubota, 2007).

A basic SPR immunosensor consists of a light source, a detector, a transduction surface, a prism, biomolecule (antigen or antibody) and a flow system. Commonly, a thin gold film with thickness from 500 to 1000 Å is deposited on a glass slide, then optically coupled to a glass prism through a refractive index matching oil. Plane polarized light is directed through a glass prism to the gold/solution dielectric interface over a wide range of incident angles and the intensity of the resulting reflected light is measured against the incident light angle with a detector. A minimum in the reflectivity is observed at which the light waves are coupled to the oscillation of surface plasmons at the gold/solution interface. An SPR angle is the angle at which the minimum in reflectivity occurs is determined, the critical angle which is very sensitive to the dielectric properties of the medium adjacent to the transducer surface apart from its dependence on the wavelength and polarization state of the incident light (Dutra et al., 2007). Fig. 8 represents a schematic view of the SPR immunoassay technique.

The first SPR immunoassay was proposed by Liedberg et al. (1983) by letting silver surface absorbing a water solution of antibody human γ-globulin (IgG) which is specifically bind with the

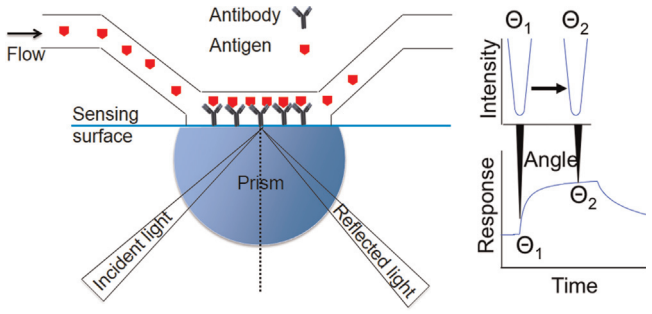

Fig. 8. Basic setup for surface plasmon resonance. Changes in the refractive index are shown with changes in the angles. Changes in the optical waves are indicated.

antihuman γ-globulin (a-IgG). It was to determine a-IgG as low as 0.2 mg/L (Liedberg et al., 1995). Similarly, for cardiac troponin detection, the first SPR immunoassay reported and developed by Wei et al. (2003), a novel label-free sandwich immunosensing method for measuring cTnI by using three monoclonal antibodies (MAbs 9F5, 2F11, and 8C12), generated by hybridoma technique and characterized by a SPR biosensor. It is using avidin as an intermediate layer and biotinylated-2F11 as the capturing antibody. Two detection methods for cTnI with the immunosensor were performed: (1) the direct detection of cTnI with a detection range of 2.5–40 μg/L and (2) the sandwich immunosensing method, in which the second antibody 9F5 biologically amplified the sensor response. As a result, the sandwich assay showed a sensitivity of 0.25 μg/L and a detection range of 0.5–20 μg/L with within-run variation of 4.9–6.7% and between-run variation of 5.2–8.4%. This method has greatly enhanced the sensitivity for detection compared to that previously reported in the literatures.

A year later, Masson et al. (2004) developed a sensor to detect markers of cardiac muscle cell death at less than 3 μg/L and less than 10 min. This fiber-optic-based SPR sensor is applied to detect cTnI. Antibodies specific to antigen of interest are attached to a carboxymethylated dextran layer on a gold SPR surface. A cTnI lower detection limit of 1.4 μg/L was achieved in preliminary tests. cTnI levels are in the range of 1–3 μg/L in patient blood after myocardial damage.

Dutra and Kubota (2007) developed a quick detection SPR immunosensor of human cTnT in real time by inventing streptavidin terminated self-assembled monolayer (SAM), which was used to bind biotinylated anti-cTnT monoclonal antibodies. With a linear range from 0.03 to 6.5 μg/L the cTnT was determined. Corresponding to a resonant angle change of 1.28 mili-degrees, the limit of detection for the SPR immunosensor was 0.01 μg/L. The system presented good repeatability with 3.4% of variation between run after regeneration of the coated surface with a solution of 1% sodium dodecyl sulfate (SDS). The sensor was also able to measure cTnT without human serum dilution with good specificity and reproducibility, practical and offered a quick response at 800 s interval.

Few months later, Dutra et al. (2007) developed a SPR sensor on a commercially available SPR AUTOLAB SPIRIT® to detect the biomarker in real time. The surface of a gold substrate via a SAM of thiols by using cysteamine-coupling chemistry is where cTnT receptor molecule was covalently immobilized. The SPR sensor presented a linear response range for cTnT between 0.05 and 4.5 μg/L ($r=0.997$, $p \ll 0.01$) with a good reproducibility (CV=4.4%). This SPR sensor has opened new perspectives of using SAM to develop regenerable immunosensor (by using a solution of 1% (w/v) SDS) with a good reproducibility allowing its use in the clinical applications.

Years later, Liu et al. (2011) employed a SAM with high antifouling ability consisting of a homogeneous mixture of oligo

(ethylene glycol) (OEG)-terminated alkanethiolate and mercaptohexadecanoic acid (MHDA) on Au for immobilizing cTnT antibody and applied in detecting cTnT by using SPR. The mixed SAM showed no phase segregation and exhibited human serum albumin resistance, particularly with an antibody-immobilized surface. X-ray photoemission spectra revealed that the chemical composition ratio of OEG to the mixed SAM was 69% and the OEG packing density was 82%. The specific binding of troponin T on the designed surface indicated a good linear correlation ($r=0.991$, $p<0.0009$) at concentrations lower than 50 mg/L with the limit of detection of 100 μg/L. The mixed SAM has the high detection capability, high accuracy and reproducibility, as well as showing strong potential to be applied in the rapid clinical diagnosis for label-free detection within 2 min.

Unlike many other immunoassays, such as ELISA, an SPR immunoassay is label free in that a label molecule is not required for detection of the analyte (Rich and Myszka, 2007), thus reducing the number of steps, permitting real time-analysis of interaction, regeneration of the sensor surface and low cost analysis (Dutra et al., 2007). SPR immunoassay can also be performed in array technique, which has the ability to be used with high-throughput and low cost method (Houngkamhang et al., 2013). Although, there are no reports related to cardiac biomarker detection using SPR immunosensor in array technique so far, this method offered an advantage of testing parallel which several samples, in a single run (Houngkamhang et al., 2013). Thus, it has opened a new possibility for multiplexed detection of cTnI and cTnT.

3.6. Colorimetric detection

Colorimetric detection is another class of optical biosensor in which chromogenic dyes is used to recognize the target (Qureshi et al., 2012). The existence of target molecules is presented by the intensity of the color changes. The limit of detection for this method can be narrowed down to a single molecule detections, thus considered as sensitive technique (Fan et al., 2008).

Wu et al. (2010) had developed a Poly(dimethylsiloxane) (PDMS)–gold nanoparticles (AuNPs) composite film-based biosensor coupled with silver enhancement colorimetric detection for cTnI as in Fig. 9. A monoclonal antibody against cTnI was immobilized on the PDMS–AuNPs composite film, followed by blocking solution and cTnI. The AuNPs act as a catalyst during the reaction of silver reduction, and this catalytic ability could be inhabited when there were proteins covering the surface of AuNPs, which influenced the amount of silver metal reduction and led to the color difference in the reaction wells. AuNPs are established as a good substrate to be functionalized with antigen, enzymes, and other biomolecules while PDMS has very good transparency, outstanding elasticity, good thermal and oxidative stability and ease of fabrication and sealed with different materials. The PDMS–AuNPs composite film has the shelf life up to several month thanks to polymer matrix of PDMS which can protect AuNPs from aggregation. The biosensor has a detection limit down to 0.01 μg/L in less than 20 min. It has low cost since it eliminates the need for expensive, highly specialized equipment.

3.7. Point-of-care assays

Although, there are currently many kinds of immunodetection methods used for cTnI and cTnT detection as mentioned above, these approaches are time consuming and usually require labeled reagents and bulky instrumentation (Bhalla et al., 2012). For these reasons, a number of POC assays based on ELISA, fluorescence, chemiluminescene, and other technologies have been developed to support the diagnosis of MI. It is generally required and expected that quantitative measurement of cTnI or cTnT should be provided and sensitivity of the POC systems should not deteriorate from result provided by automatic platforms in the central laboratory (Bingisser et al., 2012).

cTnI and cTnT quantification of POC systems can be divided into two main types, bench-top and handheld systems (Bingisser et al., 2012). Table 2 shows the list of currently available quantitative POC systems for measuring cTnI and cTnT as reported by the manufacturers with their analytical characteristics.

However, the sensitivity of currently available POC assays for cTnI and cTnT is less compared to the central laboratory test, thus limits the potential of the POC assays for reliable diagnosis of MI (Barrett et al., 2015; Bingisser et al., 2012). The development of more precise and higher-sensitivity cTn POC assays has been motivated by the necessity of serial cTn change detection, the 99th percentile calculation more accurately, and understanding why there are patients who being identified still at risk even with the present POC assays at the time showed the stone level below the 99th percentile (Jaffe and Ordonez-Llanos, 2010). According to Jaffe and Ordonez-Llanos (2010), cTnT Roche Elecsys hs-cTnT POC assay has been labeled as high-sensitive assay with the 99th percentile value at 0.013 μg/L (13 ng/L) and CV $<10\%$. Table 3 shows the characteristic of the current and the high-sensitivity cTn assays. If the imprecision is $<10\%$, 10–20%, and $>20\%$, thus, the assays are being classified as a guideline, clinically usable, and not acceptable, respectively.

Although with the higher sensitivity POC assays are available, there are significant drawbacks need to be considered, which included user-related practical issues (i.e., training, maintenance, or accreditation) and high cost and reimbursement issues, particularly when laboratory services are run on a fee-for-service basis (Bingisser et al., 2012).

The use of POC assays differs between the ST-segment elevation

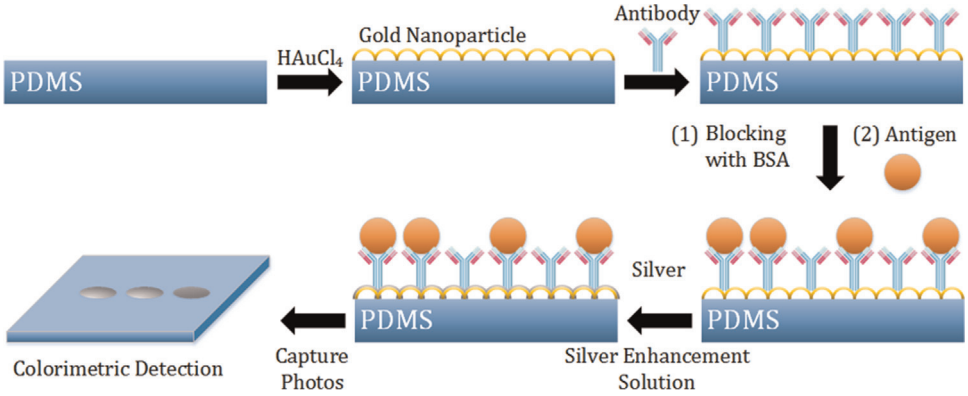

Fig. 9. Schematic diagram of the experimental procedure for silver enhancement colorimetric detection of cTnI using PDMS–AuNPs composite films biosensor.

Table 2
Cardiac troponin I and T POC assays analytical characteristics (Jaffe and Ordonez-Llanos, 2010).

Company-instrument-assay (generation)	Detection limit (μg/L)	cTn at 99th percentile (μg/L)	CV at 99th percentile (%)	cTn at 10% CV (μg/L)
Abbott AxSYM ADV (2nd)	0.02	0.04	15	0.16
Abbott ARCHITECT	< 0.01	0.028	15	0.032
Abbott i-STAT	0.02	0.08	16.5	0.1
Beckman Coulter Access Accu (2nd)	0.01	0.04	14	0.06
bioMerieux Vidas Ultra (2nd)	0.01	0.01	27.7	0.11
Innotrac Aio! (2nd)	0.006	0.015	14 (at 19 ng/L)	0.036
Inverness Biosite Triage	0.05	< 0.05	NA	NA
Inverness Biosite Triage (r)	0.01	0.056	17	NA
Mitsubishi Chemical PATHFAST	0.008	0.029	5	0.014
Ortho Vitros ECi ES	0.012	0.034	10	0.034
Radiometer AQT90	0.0095	0.023	17.7	0.039
Response Biomedical RAMP	0.03	< 0.1	18.5	0.21
Roche E170	0.01	< 0.01	18	0.03
Roche Elecsys 2010	0.01	< 0.01	18	0.03
Roche Cardiac Reader	< 0.05	< 0.05	NA	NA
Siemens Centaur Ultra	0.006	0.04	10	0.03
Siemens Dimension RxL	0.04	0.07	20	0.14
Siemens Immulite 2500 STAT	0.1	0.2	NA	0.42
Siemens Immulite 1000 Turbo	0.15	NA	NA	0.64
Siemens Stratus CS	0.03	0.07	10	0.06
Siemens VISTA	0.015	0.045	10	0.04
Tosoh AIA II	0.06	< 0.06	8.5	0.09

myocardial infarction (STEMI) and NSTEMI patients. STEMI patients have no requirement to wait for the biomarker test result, only using symptoms and specific ECG changes for immediate referral to coronary revascularization according to the international guidelines. On the other hand, for NSTEMI patients, elevated cTnI or cTnT concentrations are crucial for the diagnosis of the NSTEMI. The availability of a sensitive and accurate POC measure of cTnI or cTnT levels at the same time of ECG recordings would reduce the time spent under diagnostic evaluation (Bingisser et al., 2012).

3.8. Aptamer

Nucleic acids ligands with high affinity to the target was identified by Tuerk and Gold (1990), through establishing in vitro screening process called "systematic evolution of ligand by exponential enrichment" (SELEX), as shown in Fig. 10. Such nucleic acids were term aptamers mean "*fit*" and "*region*" in *Latin aptus* and *Greek meros*, respectively (Song et al., 2008; MacKay et al., 2014). Researchers have gained interest in aptamers especially in the field of diagnosis and disease management applications. Aptamers are able to bind a wider variety of targets than antibodies,

Table 3
Classification of the current and the high sensitivity cTn assays according to criteria of reference (Jaffe and Ordonez-Llanos, 2010).

	99th percentile (μg/L)	Imprecision at 99th percentile (%)	Classification according imprecision	% of detectable values in reference range
Current available assays (generation)				
Abbott AxSYM ADV (2nd)	0.04	15	Clinically	< 50
Abbott ARCHITECT	0.028	15	Clinically	< 50
Abbott i-STAT	0.08	16.5	Clinically	< 50
Beckman Coulter Access Accu (2nd)	0.04	14	Clinically	50–75
bioMerieux Vidas Ultra (2nd)	0.01	27.7	Not acceptable	< 50
Innotrac Aio! (2nd)	0.015	14 (at 19 ng/L)	Clinically	< 50
Inverness Biosite Triage	< 0.05	NA	NA	< 50
Inverness Biosite Triage (r)	0.056	17	Clinically	Unknown
Mitsubishi Chemical PATHFAST	0.029	5	Guideline	< 50
Ortho Vitros ECi ES	0.034	10	Guideline	< 50
Radiometer AQT90	0.023	17.7	Clinically	< 50
Response Biomedical RAMP	< 0.1	18.5	Clinically	< 50
Roche E170	< 0.01	18	Clinically	< 50
Roche Elecsys 2010	< 0.01	18	Clinically	< 50
Roche Cardiac Reader	< 0.05	NA	NA	< 50
Siemens Centaur Ultra	0.04	10	Guideline	< 50
Siemens Dimension RxL	0.07	20	Clinically	< 50
Siemens Immulite 2500 STAT	0.2	NA	NA	< 50
Siemens Immulite 1000 Turbo	NA	NA	NA	< 50
Siemens Stratus CS	0.07	10	Guideline	< 50
Siemens VISTA	0.045	10	Guideline	< 50
Tosoh AIA II	< 0.06	8.5	Guideline	< 50
Research high-sensitive assays				
Beckman Coulter Access hs-cTnI	0.0086	10	Guideline	> 95
Roche Elecsys hs-cTnT	0.013	8	Guideline	> 95
Nanosphere hs-cTnI	0.0028	9.5	Guideline	75–95
Singulex hs-cTnI	0.0101	9	Guideline	> 95

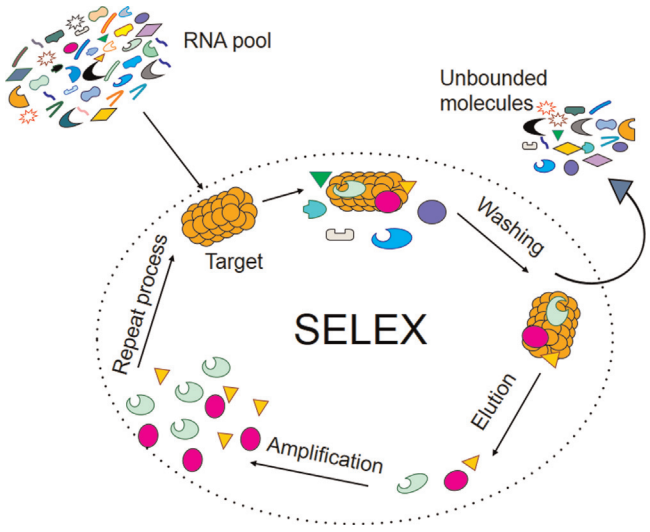

Fig. 10. The systematic evolution of ligand by exponential enrichment (SELEX) process. SELEX involves the incubation of random oligonucleotide pool with the target molecule, separation of bound from unbound nucleic acids, elution of the bound nucleic acids and amplification. Finally cloning and sequence analysis need to be done.

easier to produce and to store (MacKay et al., 2014). Even though antibodies have widely been used in biosensor developments, aptamers offer exclusive detection because of their smaller size than antibodies, stable, and capable of structural switching (Lee et al., 2008; Gopinath, 2007, 2011).

Aptamers was first reported by being used as molecular recognition element in sensor by Davis et al. (1996), when fluorescent-tagged aptamers were utilized for optical detection of human neutrophil elastase. After several years, only one research using DNA aptamer as molecular recognition for cTnI detection has been reported. Shu-hai et al. (2014) had established a new aptamer biosensor for detection of cTnI. Glassy carbon electrode (GCE) is modified with hydroxyl groups by immersing the electrode into hydrophilic solution of $NH_3 \cdot H_2O$:H_2O_2:H_2O (1:1:10) at 72 °C for 20 min. Amination solution of APTES:H_2O, as amino group, was introduced by attaching it to the surface of GCE. EDC and NHS are used for activation of the GCE electrode. Aptamer is used in the biosensor as a recognition molecule, immobilized on the surface of the GCE. Electrochemical signal is generated when the cTnI in solution is bounded to the immobilized aptamer. The current produced from the binding process is depended on the concentration of the cTnI in the solution. The linear range of the analytical signal is observed from 1.1938 μg/L to 119.3800 μg/L (lowest detection limit of 1.1938 μg/L) of cTnI in 5 min (Shu-hai et al., 2014).

4. Conclusion and perspectives

MI is the major causes of death worldwide; thus the requirement for early diagnostic is becoming increasingly important to allow initiation of lifestyle changes or appropriate medical intervention. Even though, ECG is still remained to be the recommended test to determine patient in emergency who suffered from MI, but it still lacks sensitivity. Fortunately, the finding of cardiac biomarkers as indicator for determination of MI had given hope to more accurate and sensitive result and help doctors making decision and given sufficient treatment to the patient. The cTnI and cTnT are the gold standard by cardiology consensus (i.e., Joint European Society of Cardiology – American College of Cardiology Foundation – World Heart Federation (Thygesen and Alpert, 2000; Thygesen et al., 2007) for MI detection compared to MYO and CK-MB, which also has led to the development of new devices and technologies to detect cTnI and cTnT with high sensitivity and specificity. Rapid and accurate diagnostic is main priority to facilitate more timely and efficacious intervention. A fast, accurate and ability of handling of small sample measurement for point-of-care testing of cardiac biomarkers is hence greatly desired.

The present biosensing platforms have met with these demands but needed advanced laboratory equipment and training. Thus, most of these methods have disabled the portability capability, which is required for an ideal POC lab-on-chip cardiac biomarker detection. Portability and disposability have huge applications, which include fast, simple, inexpensive POC lab-on-chips. The commercialized POC assay such as Abbott i-STAT and Roche Cardiac Reader offers portability but the limit of detection is still behind the capability of laboratory instrumentation.

Early, rapid and sensitive POC testing of the disease state becomes a vital goal for clinical diagnoses because having a quantitative measurement as soon as the symptoms of MI arise would greatly help doctors in making the best decision based on patient's present condition. Elevated cardiac troponin concentration are important for the diagnosis of NSTEMI because, the added value of a rapid POC test whether NSTEMI rule-in or rule-out is determined. Lower-risk patients with NSTEMI needed to be examined within 48–72 h. It has become a problem because it is time consuming and overcrowding the emergency department. With the availability of a sensitive and accurate point of care for cardiac troponin detection levels, would have a considerable impact to the waiting time, thus reduce the time consumption for patient in the emergency department and help doctors to act according to the patient's current condition.

To assist the doctor in making a decision in an accurate and rapid manner, an analysis of multiple biomarker simultaneously with fast and rapid speed which is capable of monitoring change of the biomarker level in a short period is highly desirable. Multiple biomarker detections can be achieved by embedding the POC device with microfluidic which guided the blood sample containing cardiac biomarkers to flow through several sensing areas of the POC. This multiplexed detection as presented by Zhang et al. (2009b) can reduce the sensing time different number of cardiac marker by combining several cardiac biomarkers into a POC device and also reduce the blood sample consumption.

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