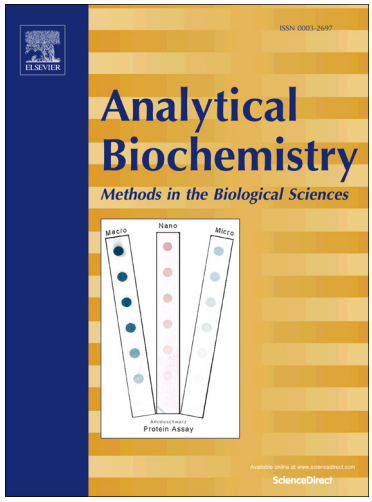

Review

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Development of optical biosensor technologies for cardiac troponin recognition

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ABSTRACT

Acute Myocardial Infraction (AMI) is the leading cause of death among cardiovascular diseases. Among the numerous attempts to develop coronary markers concept into clinical strategies, cardiac Troponin is known as a specific marker for coronary events. The cardiac Troponin concentration level in blood has been shown to rise rapidly for 4-10 days after onset of AMI and make it as an attractive approach for a long diagnosis window for detection. The extremely low clinically sensing range of cardiac troponin levels consequently makes the

methods of detection to be highly sensitive. In this review, by taking into consideration optical methods applied for cardiac troponin detection, we discuss the most commonly utilized methods of optical immunosensing and provide an overview of the various diagnosis cardiac troponin immunosensors that have been employed for determination of cardiac Troponin with considerable characters over the last several years.

Keywords: Optical Biosensor; Cardiac Troponin; Acute Myocardial Infraction

Content

1. Introduction

Biosensors are designed to detect target molecules in medical diagnostic procedures [1-3]. In principle, they are generally fabricated by immobilizing the biological elements for instance, antibody, DNA or RNA and enzyme on the surface of a transducer to convert the

interaction between biological elements and targets molecules into quantifiable signals [4-8]. Due to the high affinity of antibodies to their target molecules, antibody and antigen binding are supposed to be practical methods for detecting of a specified biomarkers in human samples [9]. Cardiovascular biosensors are classified as an extremely important and crucial diagnosing system not for only patient survival but also reduction in cost and great deal of time in successful prognosis of the disease. Acute myocardial infraction (AMI) has been remained as a leading cause of morbidity and mortality worldwide [10]. Among many biomarkers, earliest biomarkers for the detection of myocardial ischemia included aspartate aminotransferase, total lactate dehydrogenase and lactate dehydrogenase isoenzymes[11]. The other cardiac biomarker involved creatine kinase (CK), is a cytosolic carrier protein for high-energy phosphates [12].

Creatine kinase MB (CK-MB) is an isoenzyme of creatine kinase that is most abundant in the heart. CK-MB is present in a small fraction in other organs such as the small bowel, uterus, prostate, and diaphragm [13]. Therefore, the specificity of CK-MB can be reduced. The specificity comparison between CK-MB and other cardiac biomarkers including troponin showed that troponin (I or T) has been determined nearly absolute myocardial tissue specificity for myocardial damage and it has high clinical sensitivity for myocardial ischemia [14, 15]. Troponin is known as a complex of three regulatory troponin C, troponin I, and troponin T proteins, that are related to skeletal and cardiac muscle contractions. Troponin complex form can perform as a receptor of calcium ions to induce structural changes through actin and myosin providing contraction [16, 17]. The troponin complex includes three subunits: troponin C, which binds calcium; troponin I, which inhibits actin-myosin interactions; and troponin T, which attaches the troponin complex by binding to tropomysin, and facilitates contraction. Troponin C

is expressed by cells in both cardiac and skeletal muscle. In contrast, the amino acid sequences of troponins I and T are unique to cardiac muscle [18].

Troponin I (cTnI) and troponin T (cTnT) are biomarkers for AMI diagnosis. Cardiac troponin (I and T) have been recommended for predicting and treating CVD specially AMI because of their high sensitivity and specificity. Cardiac troponin I (cTnI) is known as a specific marker for coronary events [19]. Cardiac troponins as regulatory proteins control the calcium-mediated interaction of actin and myosin, which results in contraction and relaxation of striated muscle. Serum troponin levels in patients who are free of heart disease are very low or undetectable. After the AMI symptoms, the level of troponin increases in 2-4 hour and could be lift up to 14 days after acute myocardial infarction [20]. Following the myocardial damage, the troponin complex is broken up and the individual protein components are released into the bloodstream. Detection of cTnI and cTnT in peripheral blood indicates cardiomyocyte damage. As AMI is the most important cause of cell damage, both intracellular located cTns have become an integral part in the diagnosis of AMI. For this indication, determinations of cTns concentration and release kinetics are superior to all other biomarkers. Therefore cTnI and cTnT are the preferred marker for the diagnosis of AMI. However, cTn indicates and provides an estimate of cardiomyocyte damage irrespective of its cause. Therefore differential diagnoses of elevated cTns are to be considered. Electrocaediography (ECG) and cardiac troponin (cTn) form the diagnostic cornerstones and complement clinical assessment for patients with acute chest pain, angina pectoris, or other symptoms suggestive of acute myocardial infraction. The troponin I clinically sensing ranges are extremely low and the methods for detection are demanded to be highly sensitive [21-24]. Therefore, the diagnostic sensitivity of troponin compared to other tests would be extremely high. Electrocardiography (ECG) and cardiac troponin (cTn) form the

diagnostic cornerstones and complement clinical assessment for patients with acute chest pain, angina pectoris, or other symptoms suggestive of acute myocardial infraction.

In this review, we summarize major advances and developments of optical biosensors technologies for detection of cardiac troponin I (cTnI) in biological fluids. In order to achieve the increasing demand of quick troponin diagnosis and consequently the clinical therapeutics, a plethora of methods have been used for detection of troponin family, such as immuno enzymometric assay (ELISA) [25], chemiluminescent immunoassay [26], fluoro-immuno assay [27], electrical detection [28], surface plasmon resonance detection (SPR) [29], colorimetric protein array [30] and so on. These optical biosensors for cardiac troponin detection in comparison with electrochemical biosensors for troponin detection are more bulky, expensive and require difficult labelling procedures. For instance, colorimetric, fluorescence and luminometric type of sensors involve difficult labeling procedures based on indirect indicator signal schemes. The significant merits of optical biosensors are high sensitivity and rapid antigen detection.

It is worthwhile to consider the challenges in cardiac troponin detection biosensors like improving sensitivity, specificity, low cost, low power, ease of miniaturization and point of care capability [31, 32].

2. Optical biosensors

In optical biosensors, the transducing structure is the critical part and different transducers can be used for generating an optical change, e.g., grating couplers [33] resonant mirror [34] [35], surface plasmon resonance (SPR) [36], interferometry [37] reflectrometric

interference spectroscopy [38], ellipsometry [39] and total internal reflection fluorescence (TIRF) [40].

Surface plasmon resonance (SPR) technique is based on measuring the refractive index of very thin layers of material adsorbed on a metal. In SPR immunosensing, the antibodies have been immobilized on the surface of a thin metal film, as gold, deposited on the reflecting surface of a glass prism. When interactions between the antigens and immobilized antibodies occur, a change in the refractive index as variation in light intensity reflecting from the back of the film will be detected [41] [42] [43].

Localized surface plasmon resonance (LSPR) is a powerful technique for chemical and biological detection. Localized surface plasmon resonance (LSPR) is generated by light when it interacts with conductive nanoparticles (NPs) that are smaller than the incident wavelength. In localized surface plasmon resonance, by incidence of light to the nanoparticles the electric field deposited to collectively excite electrons of a conduction band, the result is coherent localized plasmon oscillations with a resonant frequency and is affected by the composition, size, geometry, dielectric environment and separation distance of NPs [44].

The generation of a guided mode in the SPR planar substrate improve the performance of SPR sensors. Plasmon-waveguide resonance is based on the deposition of a dielectric layer over a gold or silver film. For this purpose, many conductive or dielectric materials have been used such as silica and titanium dioxides. Guided modes are highly sensitive to changes in the refractive index with both polarizations [45, 46]. Fig. 1 shown the schematic of the plasmon-waveguide resonance biosensor [45].

(Figure 1)

Various integrated optical SPR sensors using slab waveguides, channel waveguides and even more waveguide structures have been developed. Fig. 2 shown the schematic SPR method in optical biosesnsor [47].

(Figure 2)

Optical biosensors are based on the alteration in the phase, polarization or frequency of the input light that related to biorecognition processes. Optical biosensors are classified into different categories, such as colorimetric, fluorescence, luminescence and surface Plasmon resonance (SPR) [48]. In biosensors based on colorimetric or fluorescence detection, the target or biological elements are labeled with fluorescent tags or dyes [48]. The presence of target molecules are determined when the change in intensity of fluorescence or color signal occurred. Colorimetric measurement is directly visible and most convenient whereas, the sensitivity is much lower. In general, biosensors based on these methods have shown effective performance but the demerits of sensitivity, miniaturization and cost efficiency. Surface plasmon resonance (SPR) can be used to detect interaction between biological elements which are immobilized on the metal surface and its bio specific target [49]. SPR immunosensors were proposed for sensitive and quick detection of human troponin. Recently, biosensors based on luminescence methods have been developed for cardiac biomarker detection [50, 51] and they have been categorized into two types, namely chemiluminescence and electroluminescence.

The limit of detection is known as the lowest concentration of troponin that can be reliably differentiated. Also, assays that have a lower limit of detection are considered more sensitive but not necessarily more precise. The limit of quantification is the lowest concentration that can be reliably detected and produce an acceptable precision and consequently, the limit of quantification may be equivalent to the limit of detection or can be at a much higher

concentration [52]. The limit of quantification for troponin assay is the concentration with a total imprecision (CV) of 10%. The importance of cardiac specific troponin is that even small amounts of the cardiac specific troponin play a critical role to reflect incremental risk and indicate myocardial injury [53].

2.1. Fluorescence-based biosensors for cardiac Troponin detection

Several immunoassay techniques may be used to monitor cTnI. Fluorescence based biosensors have developed to detect troponin I also For troponin I detection, fluoro-microbead guiding chip (FMGC) performing by an optical immunoassay, has been reported [54].

In 2011, Seung Yeon Song et al. have developed an optical immunoassay for cardiac Troponin I. Antibody-tagged fluoro-microbeads have been used to perform a sandwich immunoassay. The target antigen cTnI was added to cTnI capture antibody immobilized on the DTSP (3-3 Dithiobis-propionic acid N-hydroxysuccinimideester)-functionalized surface and then antigen antibody bind. Biotin –conjugated cTnI detection antibody was loaded into chip and reacted for 30 min. Immobilized cTnI bind to fluoro-microbead conjugated antibody. The microchanel was washed with PBS and then avidin-conjugated fluoro-microbeads were injected. The bound fluoro-microbead conjugates have been measured directly using a conventional fluorescence microscope. Fig. 3. shown design and schematic diagram of the sandwich assay on fluoro-microbeads guiding chip [54].

(Figure 3)

2.2. Luminescence and colorimetric methods for cardiac Troponin detection

Because of the need to fast diagnosis for clinical therapeutics, biosensors have been designed to have small size and weight, fast response time, high sensitivity and importantly ease of operation and fabrication [55, 56]. For calorimetry assay, materials such as silicon chips [57] , glass surfaces [58] and gold electrodes [59] have been developed. In 2010, Wen-YaWu et al. have proposed biosensor based on colorimetric method for cardiac troponin I detection. They showed that Poly(dimethyl siloxane) PDMS-gold nanoparticles (Au NPs) composite film as basis with silver enhancement can be employed by colorimetric method for detection of cardiac troponin I(cTnI) [60]. PDMS has merits such as excellent transparency, outstanding elasticity, good thermal and oxidative stability, ease of fabrication and ability to be sealed with various materials. Gold nanoparticles can be functionalized as an idle substrate with antigen, enzyme and other biomolecules. Hence, applying Au NPs patterned on PDMS films has the most advantage in biosensor applications. Au NPs aggregate in liquid system therefore, Au NPs have immobilized firstly on to PDMS film and then covered with antibodies. After blocking the surface with BSA (Bovine Serum Albumin) and capturing cTnI, silver enhancement solution were dropped onto surface of sensor and took live photos. Au NPs play the role of catalyst during reactions of silver reduction and when protein covered the surface of NPs, the catalytic property of Au NPs could be wasted. Differences in the type, quality and quantity of proteins that covered surface of Au NPs influence the mount of reduction silver metal and cause the color differences of reaction mixture. The detection of cardiac troponin I (cTnI) using PDMS-Au NPs composite film is practically used for clinical diagnosis. Experimental procedure and schematic diagram of silver enhancement colorimetric detection of cardiac troponin I have shown in Fig. 4. [60]

(Figure 4)

Chemiluminescence (CL) and electroluminescence (ECL) biosensing platform have been assessed for cardiac markers detection [61, 62]. A novel nanoparticle based on electrochemiluminescence (ECL) immunosensor has been employed for sensitive detection of human cardiac troponin I (cTnI) [22, 63]. The production of luminescence during electrochemical reaction is considered as elecrochemiluminescence (ECL). High sensitivity, wide linear, low background and simple instrument which can be used for detecting the acute myocardial infarction biomarkers are taking into account as the positive characteristics of ECL. The unique property of nanoparticles in coupling with biomolecules is an important issue for developing biological nanoprobes [64]. Au NPs have been functionalized by protein via the electrostatic and other physical adsorption mechanisms and due to high surface to volume ratio of Au NPs, biomolecules can be immobilized to surface of them. Gold nanoparticles were functionalized by using N-(aminobutyl)-N-(ethylisoluminol)(ABEI-Au NPs) and are used as labels via simple seed growth method for ultrasensitive detection of cTnI in clinical diagnosis [63]. Two kinds of nanoparticles including SA-Au NPs (streptavidin coated gold nanoparticles) and ABEI-Au NPs-Ab_2 nanoprobes were used for ECL immunosensor. SA-Au NPs enhance the conductivity or electron transfer between biomolecules and electrode and improve immobilized capacity. There are two advantages in application of Au NPs in ABEI-Au NPs- Ab_2 nanoprobes. First, Au NPs carry antibodies for recognition of biomarkers and Second, numerous ECL signal generating molecules ABEI were immobilized on surface of them for signal amplification. The biotinylated-Ab_1 immobilized on the surface of SA-Au NPs/ 1,3-propanedithiol/Au, antigen

cTnI, and ABEI –Au NPs-Ab_2 nanoprobes fabricate sandwich type ECL immunosensor. Design and fabrication process are shown in Fig. 5.

(Figure 5)

According to Fig. 5 when the ABEI-Au NPs-Ab_2 probes were reacted with Ag/BSA/bio-Ab1/SA-AuNPs/1,3-propanedithiol/Au substrate, the strong ECL signals were obtained which is attributed to the ABEI-AuNPs-Ab2 molecules attached on the electrode surface and is the result of the reaction of ABEI radicals electro-oxidized by ABEI with H_2O_2 beside the catalysis of AuNPs.

In 2013, Fang Li et al. indicated the possibility of sensitive label–free ECL immunosensor employed by using luminol functionalized gold nanoparticles (luminol-Au NPs) as antibody carriers for detection of the cTn I acute biomarker [22]. Luminol-Au NPs conjugated biotinylated antibodies against cTnI (biotin-anti-cTnI-luminol-AuNPs) was assembled on to a streptavidin coated Au NPs (SA- Au NPs) modified electrode.

Luminol molecules functionalized surface of electrode for generating ECL signals and they carried numerous biotin-anti-cTnI for connecting with the SA-Au NPs modified electrode and recognition of target cTnI. The fabrication process of the label free ECL immunosensor using luminol –Au NPs have shown in Fig. 6.

(Figure 6)

The ECL reaction of luminol with H_2O_2 formed electro-transfer interactions. In the presence of Au NPs as the catalyst and absence of cTnI, luminal anions and HO_2^- in alkaline

solution were electro-oxidized to luminol radicals ($L^{\cdot-}$) and ($O_2^{\cdot-}$) radicals. In the presence of cTnI, biotin-anti-cTnI-luminol- Au NPs assembled Au electrode captured the target cTnI and immune-reaction happened. The ECL intensity is dependent on concentration of cTnI [22].

2.3. SPR-based biosensor for cardiac Troponin detection

SPR immunosensing includes a thin metal film deposited on surface of glass prism and biorecognition elements containing of proteins/antibodies/DNA/RNA have been immobilized on the surface of sensor [65]. When antigen reacting with biorecognition element, refractive index as variation in light intensity changed [66].

Surface plasmon resonance (SPR) technic has been used for analytical application in immunosensors [67]. The high sensitivity to the variation of mass on the transducer surface in this technic resulted the refractive index alteration By immobilizing antibodies in immunosensors [68].

In 2007, Rosa Fireman Dutra et al. have developed covalently immobilized antibody against cTnT on gold surface via a self-assembled monolayer (SAM) of thiols by using cysteamine-coupling chemistry [69]. Immobilization methods are one of the most imperative aspects for developing of immunosensors.

SAMs have shown some advantages such as high stability, acceptable orientation and easy preparation and it became particular method to immobilize biological elements for biosensors development. Mercapto groups attached rigidity versatile SAM to the gold electrode surface [69]. The amino groups of cysteamine SAM via cross-linking by glutaraldehyde

immobilized antibodies on surface of sensor. Schematic of SPR immunosensor for cTnT detection have shown in Fig. 7.

(Figure 7)

In this study, by a SPR software (4.1.2 version from Eco chemie) the response of SPR sensor was automatically monitored and Antibodies were immobilized covalently via use of SAM and they allowed repetitive measurement with cost saving [69].

In 2007, Rosa Fireman Dutra and Lauro Tatsuo Kubo have proposed an SPR sensor that streptavidin was immobilized on a replaceable carboxy methyldextran hydrogel, which was used for binding biotinylated anti troponin T monoclonal antibodies to detect human cardiac troponin T [70]. SPR sensor component involve a gold- coated glass disc covered covalently with a carboxymethylated dextran.

The dextran matrix by the functionality of the surface elevates the binding capacity of the surface that streptavidin ligand via amino groups is coupled to dextran. Biotinylated anti troponin T monoclonal antibodies interacted with streptavidin coated sensor disc and monitored by observing changes in the resonant angle [70]. The interaction between biotinylated mab cTnT and streptavidin is shown in Fig. 8.

(Figure 8)

The non-specific binding on the surface is one of the major problems in this method and it can make changing in the resonant angle. There are several ways to minimize this problem,

several washing after the antigen recognition by immobilized antibody and the blocking of free reactive sites by a selective layer [70]

A linear range of detection was from 0.03 up to 6.5 ng/ml. The system presented was possible to measure the cTnT without dilution of the human serum with good specify and reproducibility [70].

In 2011, Jen Tsai Liu et al. have investigated on Immobilized troponin T antibody via self-assembled monolayer (SAM) comprising a homogeneous mixture of oligo(ethylene glycol)(OEG-terminated alkanethiolated and mercaptohexadecanoic acid) (MHDA) on Au by using Surface Plasmon resonance (SPR). oligo(ethylene glycol) resist the adsorption of protein from solution and it has been employed for surface grafting. Because of high hydrophilicity, hydrogen bond accepter, and the zero net charge, the self- assembled monolayer (SAM) of OEG is suitable for making the anti-fouling surface. Functional group of the OEG cannot cross link with biomolecules and its anti- fouling ability is lost [71].

For this reason, OEG was combined with mercaptohexadecanoic acid (MHDA) and was created a mixed SAM. The MHDA has Carboxyl groups that can be coupled with Amine groups on the antibody through the carbodiimide hydrochloride (EDC) / N--hydroxysulfosuccinimid EDC/NHS using active sites [71].

Surface modification of the mixed SAM of OEG with MHDA and immobilization methods for binding antibody have shown in Fig. 9.

(Figure 9)

The SPR biosensor detected troponin T within 2 min after injection in the linear detection range lower than 50 μgml^{-1} and The limit of detection (LOD) of cardiac troponinT 100 ng mL−1, which is not yet sufficient for clinical diagnostics [71].

2.4. Other optical-based biosensors for cardiac Troponin detection

One of the methods for troponin detection is based on an optomagnetic immunoassay technology. This method can be used in affinity based assay for the detection of broad range of biological molecules including proteins, small molecules and nucleic acid [72-75].

Opteomagnetic biosensor based on nanoparticles that are magnetically actuated and optically detected in a sample fluid was proposed for troponin detection [76, 77]. There are electromagnet placed above and below the cartridge that generate magnetic forces and supermagnetic particles were controlled and accelerated by them [77].

D. M. Bruls et al., in 2009, coated the magnetic nanoparticles and the sensor surface with antibody and magnetic nanoparticles were mixed into sample fluid in the cartridge. Due to the large total surface area of the dispersed nanoparticles, nanoparticles captured analyte molecules [78]. Then, the electromagnets were applied to move the nanoparticles and localized them at the binding surface. For removing free and weakly bound particles from surface, magnetic field from upper magnet is used. The optomagnetic system with electromagnets and detection optics were placed in the fluid microchamber [77]. Design and assay process steps have shown in Fig. 10.

(Figure 10)

For detecting the presence of magnetic nanoparticles on binding surface, the optical principal of frustrated total internal reflection (f-TIR) [79] was employed that has shown in Fig. 10b. [77]

D. M. Bruls et al.in a study developed a fast and sensitive cTnI test based on actuated magnetic nanoparticles. For this purpose, nanoparticles that were incubated with sample fluid injected into cartridge. The actuation protocol was started and magnetic force from the lower magnet was applied to particles. Particles that carrying cTnI were concentrated at the surface and cTnI bound between antibody-coated nanoparticles and antibody-coated surface of sensor. Then, the optical signal has been changed [77].

Fluorescence resonance energy transfer (FRET) is known as a chemical transduction method that describing energy transfer between two light- sensitive molecules. Donor and acceptor molecules must be in close proximity. The absorption spectrum of the acceptor must overlap the fluorescence emission spectrum of the donor. The FRET biosensors are categorized into two types, intermolecular (or bimolecular) and intramolecular (or unimolecular) [80].

In 2008, R. Cody Stringer et al. have employed biosensor based FRET by using quantum dots as donors and organic dyes as acceptors for detection of troponin I [81]. To detect the fluorescence, a liquid core waveguide was utilized that was able to achieve highly sensitive an accurate measurement. For this purpose, green quantum dot was used as the donor with the emission peak wavelength at proximately 544 nm and Invitrogen Alexa-Fluor 546 (AF-546) fluorescent dye was used as acceptor that absorption peak wavelength is directly overlap with emission peak wavelength of donor. The amine – reactive ester on the surface of quantum dot was created by using 1-Ethyl-3-[3-dimethylamino- propyl] carbodiimide hydrochloride (EDC) and N--hydroxysulfosuccinimide (sulfo-NHS) and then protein A was labeled with carboxyl-

functionalized quantum dot. Mouse anti-Troponin I IgG monoclonal antibody was labeled with AF-546 fluorescent dye and then, the biosensor complex was fabricated. Antibodies conclude two F_{AB} arms and a single F_C stem which F_{AB} are bound to single F_C stem via means of a hinge region [82, 83]. Protein A have simultaneously able to binding two IgG antibodies via F_C portion of the IgG [84]. Quantum dot-labeled protein A and mouse anti-Troponin I IgG monoclonal antibody–labeled AF-546 fluorescent dye were incubated for 2 hours at room temperature. After that, protein A binds to anti-Troponin antibody and self-assembled optical biosensor is produced. Schematic of self-assembled optical biosensor architecture has shown in Fig. 11 [81]

(Figure 11)

In order to obtain the emission spectrum with significant peak and large changes in energy transfer, the acceptor to donor ratio was illustrated 4:1 AF-546 to quantum dot. When antibody captured antigen, conformational change occurs within antibody structure. Due to changing in distance between quantum dot as donor and AF-546 as acceptor, the energy transfer alter [81].

Surface enhanced Raman scattering (SERS) is a technique which offers orders of magnitude increases in Raman intensity, overcoming the traditional drawback of Raman scattering [85]. SERS is of interest for trace material analysis, flow cytometry and other applications where the current sensitivity/speed of a Raman measurement is insufficient. The enhancement occur at a metal surface which has nanoscale roughness, and the molecules adsorbed onto that surface can undergo enhancement [85, 86]. Typical metals used are gold or silver. Preparation of the surface can be provided through electrochemical roughening, metallic

coating of a nano-structured substrate, or deposition of metallic nanoparticles (often in a colloidal form).

One of the more recent developed techniques for immunoassay and DNA detection is surface enhanced Raman scattering [87]. This technique is in high interest due to its high sensitivity and potential for multiplexing [63, 88, 89]. Since, Raman peaks are extremely narrower than fluorescence bands, thus SERS (surface enhanced Raman scattering) has potential for multiplex detection [90]. SERS assay is used recently via SERS nano-tags and magnetic beads for a sandwich immunoassay [91, 92]. This method includes two antibodies that one of them is attached to the surface of magnetic beads and the other is conjugated with a SERS nano-tag. As a result, the antigen is sandwiched between two antibodies [93].

In 2014, Hyangh Chouand et al. have developed a SERS-based competitive immunoassay for detection of cTnI and CK-MB[93]. For this reason, monoclonal antibodies for cTnI and CK-MB were immobilized onto surface of magnetic beads and two different type of SERS nano-tags were used for conjugated target antigen (cTnI and CK-MB). Samples including free target antigens and the antigens conjugated onto the surface, SERS nano-tags were injected into magnetic beads and competitive reaction between the monoclonal antibodies on the magnetic beads and free target antigens and the antigens conjugated on the surface of SERS nano-tags started. By using a small magnetic bar, the immunocomplexs were separated and the raman signals of the remaining SERS nano-tags in supernatant were examined. In this study, two type of SERS nano-tags were utilized, malachite green isothio-cyanate (MGITC) and X-rhodamine-5-(and-6)-isothiocyanate (XRITC) were used as Raman reporter molecules. Hallow gold nanospheres (HGNs) have been used for conjugating cTnI and CK-MB antigens [93].

3. Conclusions and perspectives

The importance of cardiac Troponin biomarkers for the fabrication of immunosensors and their application in Acute Myocardial Infarction (AMI) concept are known. Fabrication and modification of immunosensors by incorporation of biological molecules via different methods have been carried out to improve the detection of specified biomarkers.

Recent advances in the optical immunosensing of cardiac Troponin biomarkers were summarized and the conception of detection and the development of devices based on optical – detection principles considered and implies that for fabrication of an ideal diagnosis CVD (cardio vascular diseases) at early stages, it is essential to detect cardiac Troponin biomarkers with simple and sensitive method at very low levels of Troponin through optical measurement in various methods such as colorimetric, fluorescence, luminescence and surface Plasmon resonance (SPR), and so on. Several disadvantages of optical immunosensors like bulky, cost and difficult labeling procedures have been proposed. Thus, clinical biosensors based on colorimetric method using PDMS-Au NPs composite film with silver enhancement have been attempted successfully for cTnI detection in less than 20 min and limit of detection of 0.01 ng/ml. Furthermore, the advantages of calorimetric method has been considered in the ease of fabrication and operation and low cost. Whereas, in surface plasmon resonance (SPR) immunosensor the detection of cTnI in a linear range from 0.03 up to 6.5 ng/ml has been reported. Also, the detection limit of the mentioned sensors was 0.01 ng/ml with the merits of good repeatability and specificity and concurrently compare to the fabrication strategy of the biosensor based on ECL immunosensors for the detection of cTnI. Moreover, ECL immunosensor by using luminal- Au NPs as antibody with wide range from 0.1 to 1000 ng/ml and limit of 0.06 ng/ml for detection have shown a great application potential in clinical and

pharmaceutical analysis and the optomagnetic biosensor technology based on actuated nanoparticles for troponin detection have proposed advantages such as high sensitivity, rapid, multiplexed assays on a small sample volume and low-cost disposable cartridge.

Finally, the precision of troponin assays improvement will be continued and ameliorate earlier diagnosis of acute myocardial infarction and better risk stratification upon use of the new highly sensitive assays. It is worth mentioning that testing for troponin has not been set the same level of precision as highly automated methods and still remains as an area for deeper understanding and efficacy evaluations and we reported that the optical biosensors with highly specific detection of troponin have the ability for more investigation in clinical applications.

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Figure captions

Figure 1. Schematic shown the Plasmon-waveguide resonance biosensor [45]

Figure 2. Schematic shown the SPR method in optical biosesnsor [47]

Figure 3. (A) Design of the fluoro-microbead guiding chip (FMGC). (B) Photograph of the FMGC. (C) 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 [54]

Figure 4. Experimental procedure for silver enhancement colorimetric detection of cardiac troponin I: (a) PDMS chip with HAuCl4 solution, (b) photo of PDMS–AuNPs composite film and (c) schematic diagram for colorimetric detection [60]

Figure 5. Schematic illustration for the fabrication process of the sandwich-type ECL immunosensor using SA-AuNPs as immobilizationmatrix and ABEI-AuNPs-Ab2 as probes [63]

Figure 6. Schematic illustration of the fabrication processes of the label-free ECL immunosensor using luminol-AuNPs as antibody carriers and sensing platform [22]

Figure 7. Schematic representation of the principle of SPR immunosensor for cTnT determinations [69]

Figure 8. Illustration of the immobilization scheme of biotinylated-mab cTnT [70]

Figure 9. Schematic diagram of surface modification for the mixed SAM of OEG and MHDA and immobilization method of antibody binding carboxyl groups of MHDA on Au [71]

Figure 10. Optomagnetic immuno-biosensor based on actuated magnetic nanoparticles. (a) Schematic shown the assay process in the reaction microchamber: (a1) antibody conjugated magnetic nanoparticles filling microchamber and capturing of analyte (a2) the electro magnets generate magnetic field that are applied on magnetic particles to binding to the surface. (a3) magnetic field from upper magnet is used to remove unbound and weakly bound particles. (b) The fluid microchamber placed in the optomagnetic system with electromagnets and

detection optics. Light reflects from the sensor surface with an intensity that depends on the concentration of nanoparticles at the surface of the sensor, with the mechanism of frustrated total internal reflection (f-TIR). (c) Cartridge is composed of two plastic parts connected by double-side adhesive tape. Outer dimension of the cartridge is 1cm × 4cm. the cartridge involves a sample inlet, a channel, a reaction microchamber and a vent. The volume of the reaction microchamber is 1 µl and a total sample volume is 10 µl. (d) f-TIR image of magnetic nanoparticles bound to the surface of the sensor through an immunoassay on 31 capture spots of 125 mm diameter each. (e) Schematic real-time curve of the measured optical signal for a single capture spot. The assay phases a1–a3 are marked. The signal modulation in phase a2 is due to switched actuation of the magnetic nanoparticles [77]

Figure 11. Schematic of self-assembled optical biosensor architecture [81]

Figures

Figure 1

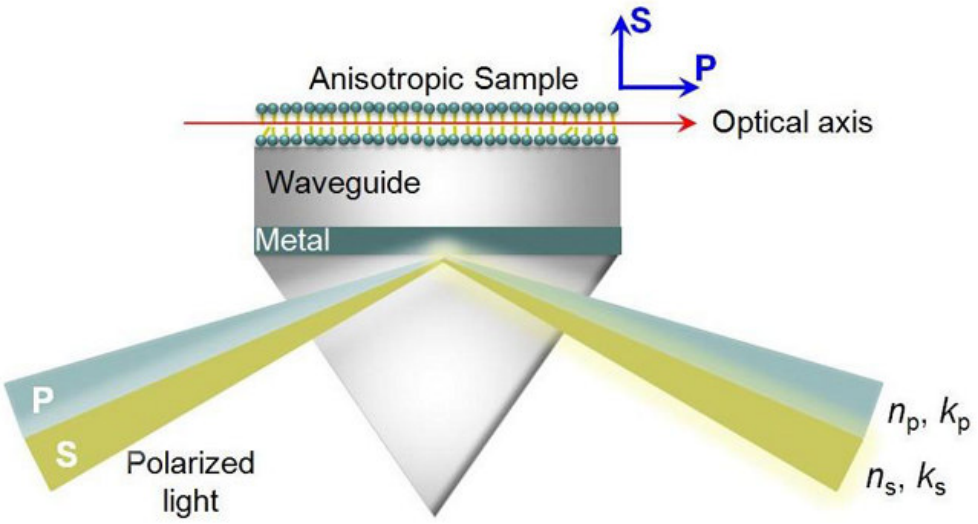

Figure 2

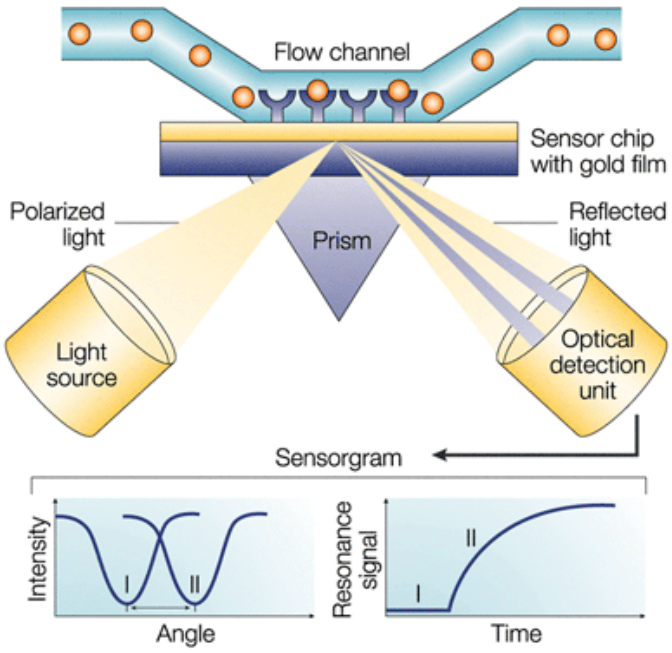

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Figure 3

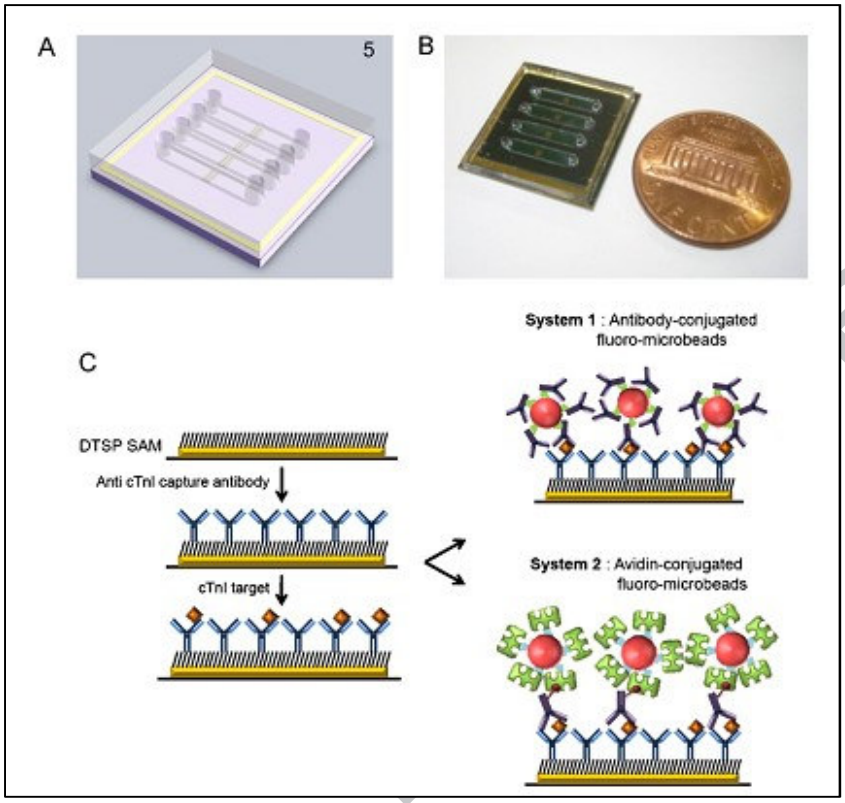

Figure 4

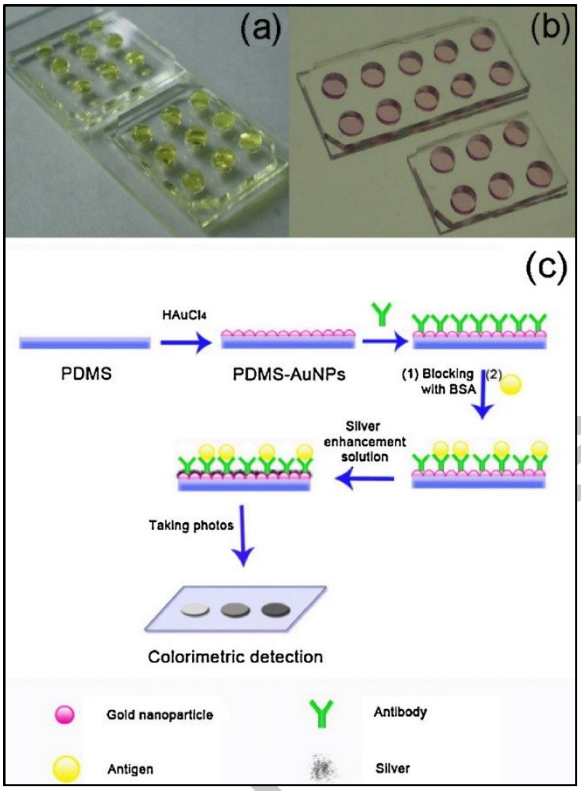

Figure 5

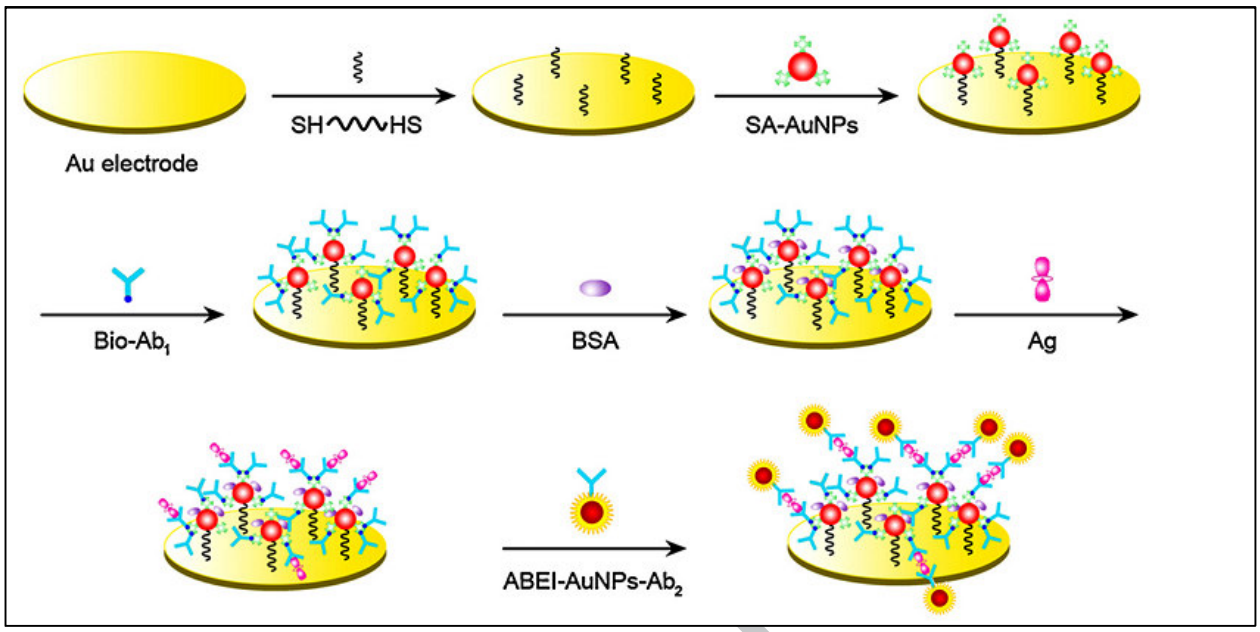

Figure 6

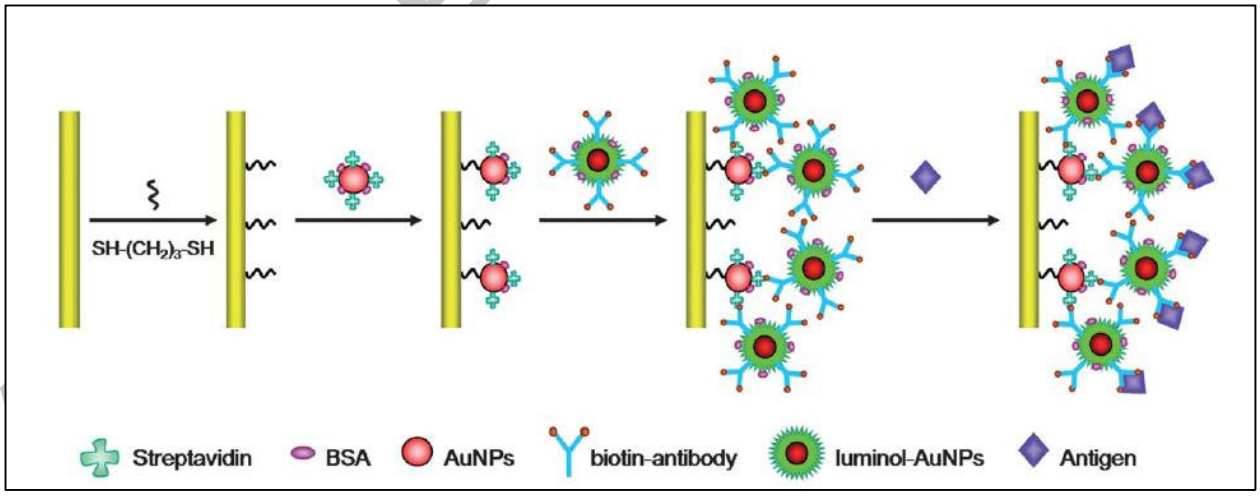

Figure 7

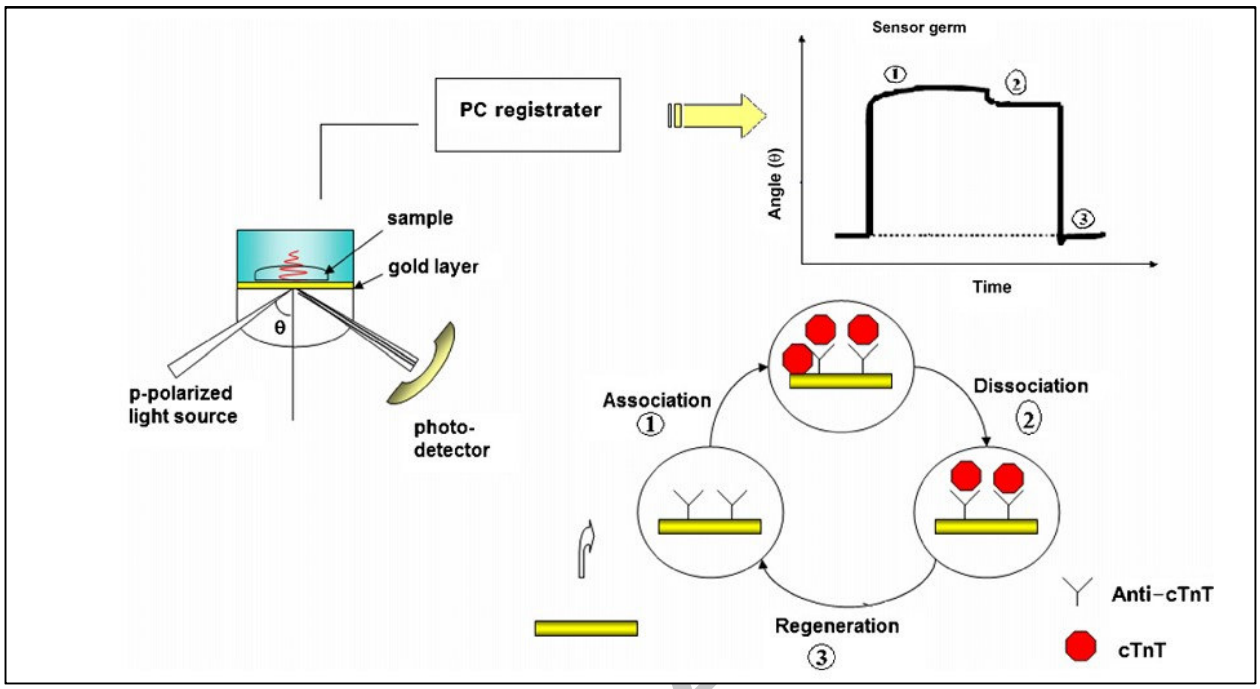

Figure 8

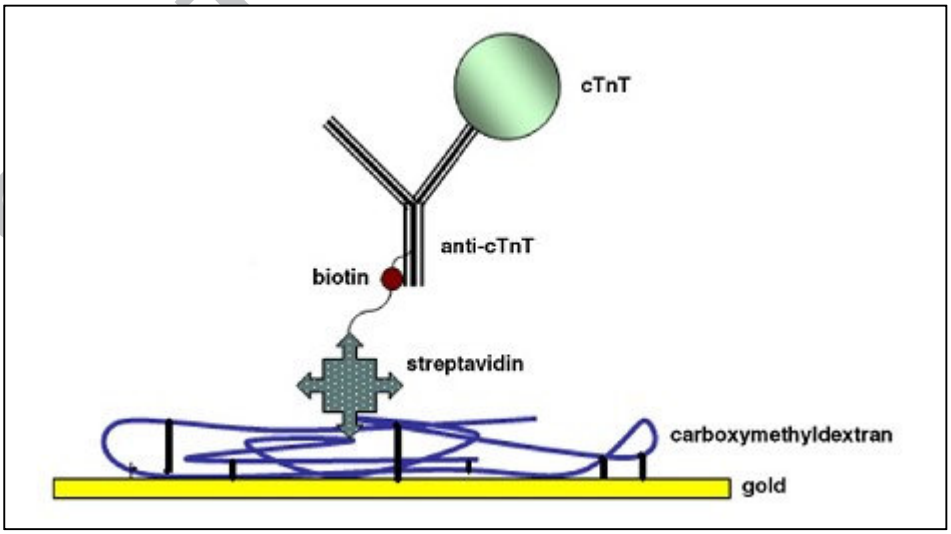

Figure 9

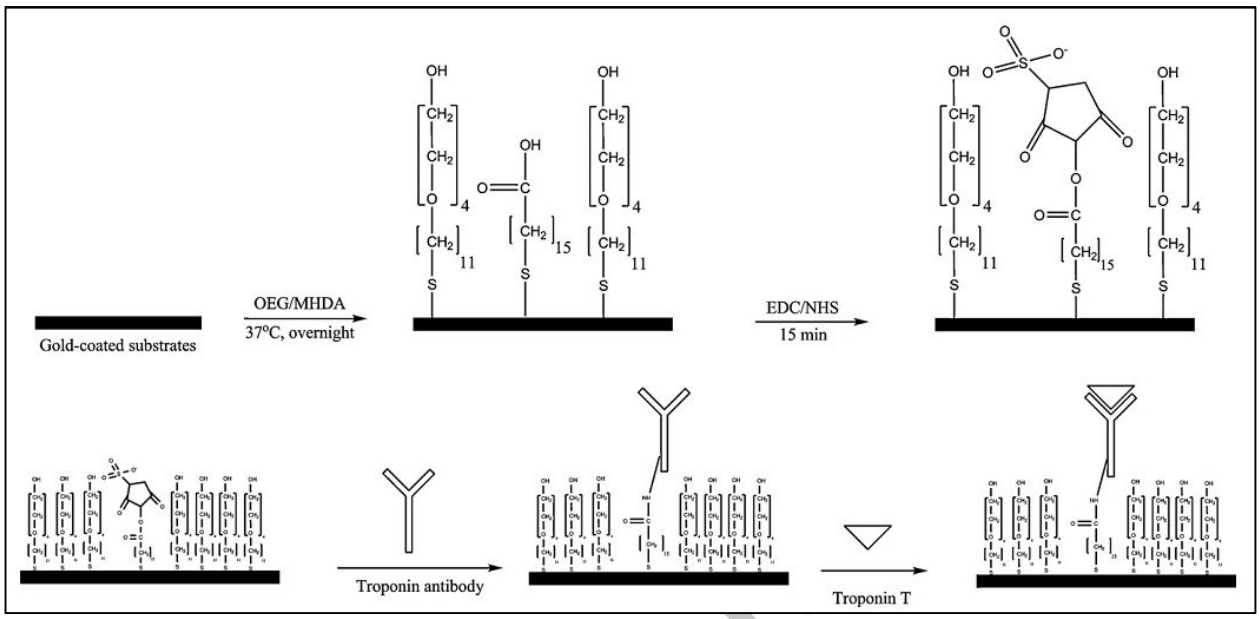

Figure 10

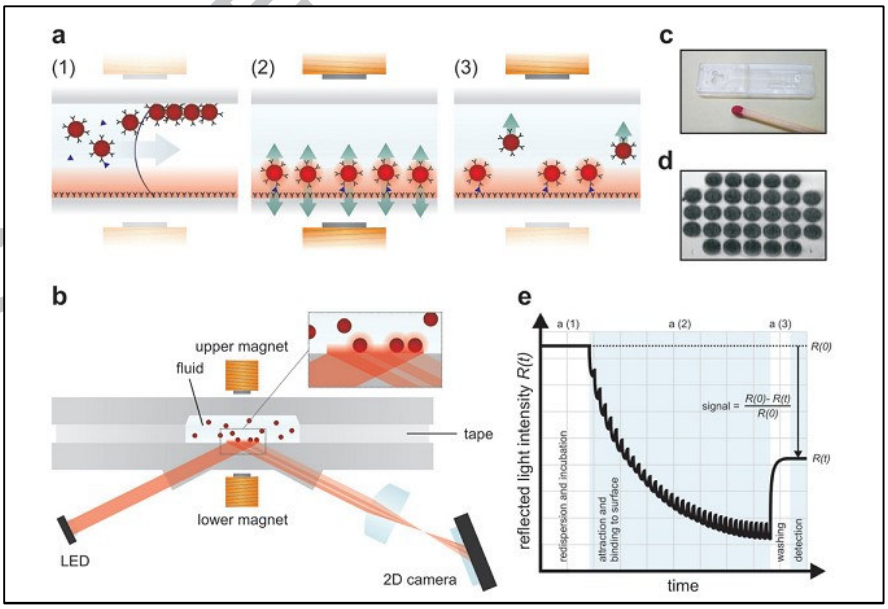

Figure 11