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Abstract: Acute myocardial infarction (AMI) is the leading cause of death among cardiovascular diseases. Among the numerous attempts to develop coronary marker concepts 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, making it an attractive approach for a long diagnosis window for detection. The extremely low clinical sensing range of cardiac troponin levels consequently makes the methods of detection highly sensitive. In this review, by taking into consideration optical methods applied for cardiac troponin detection, we discuss the most commonly used methods of optical immunosensing and provide an overview of the various diagnostic cardiac troponin immunosensors that have been employed for determination of cardiac troponin over the last several years.

Biosensors are designed to detect target molecules in medical diagnostic procedures.[1,2,3] In principle, they are generally fabricated by immobilizing the biological elements of, for instance, antibody, DNA or RNA, and enzyme on the surface of a transducer to convert the interaction between biological elements and target molecules into quantifiable signals.[4,5,6,7,8] Due to the high affinity of antibodies to their target molecules, antibody and antigen binding are supposed to be practical methods for detecting specified biomarkers in human samples.[9] Cardiovascular biosensors are classified as an extremely important and crucial diagnosing system not only for patient survival but also for reduction in cost and a great deal of time in successful prognosis of the disease. Acute myocardial infarction (AMI)[1] has

remained a leading cause of morbidity and mortality worldwide.[10] Among many biomarkers, the earliest biomarkers for the detection of myocardial ischemia included aspartate aminotransferase, total lactate dehydrogenase, and lactate dehydrogenase isoenzymes.[11] Another cardiac biomarker, 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 of other organs such as the small bowel, uterus, prostate, and diaphragm.[13] Therefore, the specificity of CK-MB can be reduced. Specificity comparison between CK-MB and other cardiac biomarkers, including troponin, showed that troponin (I or T) has been determined to have nearly absolute myocardial tissue specificity for myocardial damage and has high clinical sensitivity for myocardial ischemia.[14,15] Troponin is known as a complex of three regulatory proteins—troponin C, troponin I, and troponin T—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 has 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 tropomyosin 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]

Cardiac troponin I (cTnI) and cardiac troponin T (cTnT) are biomarkers for AMI diagnosis. Cardiac troponins (cTns) have been recommended for predicting and treating cardiovascular disease, especially AMI, because of their high sensitivity and specificity. 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 AMI symptoms, the level of troponin increases in 24 h and could increase 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. Because 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 cTn concentration and release kinetics are superior to all other biomarkers. Therefore, cTnI and cTnT are the preferred markers 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. Electrocardiography (ECG) and cTn form the diagnostic cornerstones and complement clinical assessment for patients with acute chest pain, angina pectoris, or other symptoms suggestive of acute myocardial infarction. The troponin I clinical sensing ranges are extremely low, and the methods for detection are required to be highly sensitive.[21,22,23,24] Therefore, the diagnostic sensitivity of troponin compared with other tests would be extremely high. Electrocardiography and cardiac troponin form the diagnostic cornerstones and complement clinical assessment for patients with acute chest pain, angina pectoris, or other symptoms suggestive of acute myocardial infarction.

In this review, we summarize major advances and developments of optical biosensor technologies for detection of cardiac troponin I in biological fluids. To achieve the increasing demand of quick troponin diagnosis and consequently the clinical therapeutics, a plethora of methods have been used for detection of the troponin family, including enzyme-linked immunosorbent assay (ELISA),[25] chemiluminescent immunoassay,[26] fluoroimmunoassay,[27] electrical detection,[28] surface plasmon resonance (SPR) detection,[29] and colorimetric protein array.[30] These optical biosensors for cardiac troponin detection, in comparison with electrochemical biosensors for troponin detection, are more bulky and expensive and also require difficult labeling procedures. For instance, colorimetric, fluorescence, and luminometric types 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 such as improving sensitivity and specificity, low cost, low power, ease of miniaturization, and point-of-care capability.[31,32]

Optical biosensors

In optical biosensors, the transducing structure is the critical part and different transducers can be used for generating an optical change, including grating couplers,[33] resonant mirror,[34,35] surface plasmon resonance (SPR),[36] interferometry,[37] reflectometric interference spectroscopy,[38] ellipsometry,[39] and total internal reflection fluorescence (TIRF).[40]

The 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, such 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. LSPR is generated by light when it interacts with conductive nanoparticles (NPs) that are smaller than the incident wavelength. In LSPR, by incidence of light to the NPs, the electric field is deposited to collectively excite electrons of a conduction band, and 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 improves 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, including silica and titanium dioxides. Guided modes are highly sensitive to changes in the refractive index with both polarizations.[45,46] Fig. 1 shows a schematic of the plasmon waveguide resonance biosensor.[45]
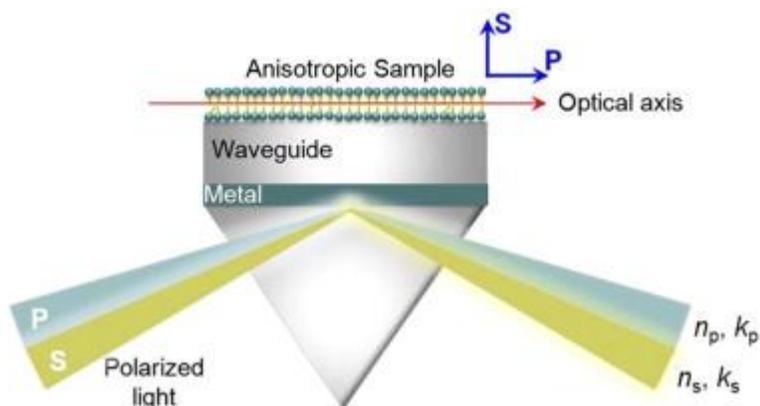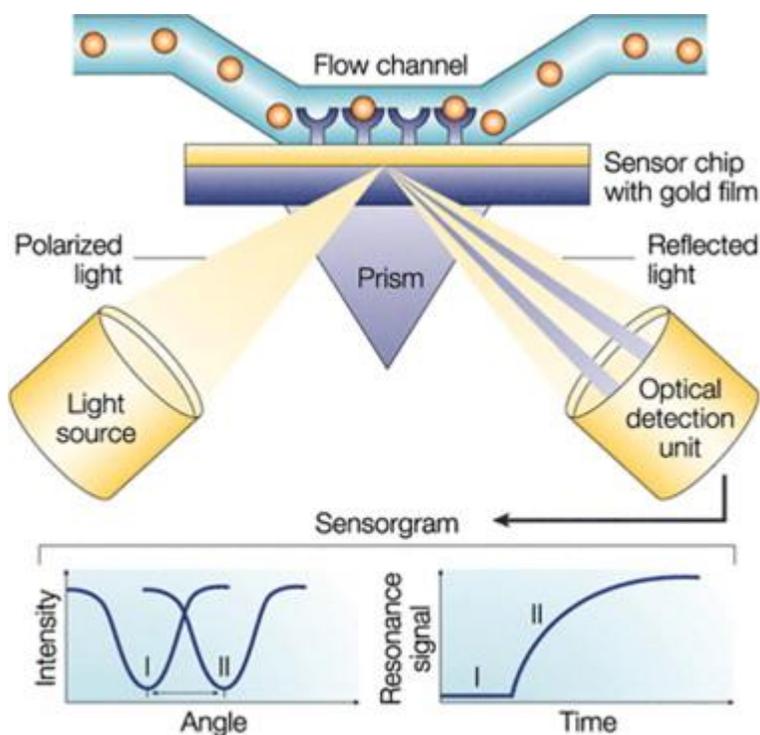
Fig.1. Schematic showing the plasmon waveguide resonance biosensor.[45]

Various integrated optical SPR sensors using slab waveguides, channel waveguides, and even more waveguide structures have been developed. Fig. 2 shows a schematic SPR method in an optical biosensor.[47]

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Fig.2. Schematic showing the SPR method in optical biosensor.[47]

Optical biosensors are based on the alteration in the phase, polarization, or frequency of the input light that is related to biorecognition processes. Optical biosensors are classified into different categories such as colorimetric, fluorescence, luminescence and surface plasmon resonance.[32] In biosensors based on colorimetric or fluorescence detection, the target or biological elements are labeled with fluorescent tags or dyes.[32] The presence of target molecules is determined when the change in intensity of fluorescence or color signal occurs. Colorimetric measurement is directly visible and convenient, whereas the sensitivity is much lower. In general, biosensors based on these methods have shown effective performance, but the demerits are sensitivity, miniaturization, and cost efficiency. SPR can be used to detect interaction between biological elements that are immobilized on the metal surface and its biospecific target.[48] SPR immunosensors have been proposed for sensitive and quick detection of human troponin. Recently, biosensors based on luminescence methods have been developed for cardiac biomarker detection[49,50] and 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. In addition, 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 can 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.[51] The limit of quantification for troponin assay is the concentration with a total imprecision (coefficient of variation, CV) of 10%. The importance of cardiac-specific troponin is that even small amounts of cardiac-specific troponin play a critical role in reflecting incremental risk and indicating myocardial injury.[52]

Fluorescence-based biosensors for cardiac troponin detection

Several immunoassay techniques may be used to monitor cTnI. Fluorescence-based biosensors have been developed to detect troponin I as well. For troponin I detection, fluoro-microbead guiding

chip (FMGC) performing by an optical immunoassay has been reported.[53]

In 2011, Song and coworkers developed an optical immunoassay for cTnI. Antibody-tagged fluoro-microbeads were 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*-hydroxysuccinimide ester)-functionalized surface and then antigen antibody bound. Biotin-conjugated cTnI detection antibody was loaded into the chip and reacted for 30 min. Immobilized cTnI bound to fluoro-microbead-conjugated antibody. The microchannel was washed with phosphate-buffered saline (PBS), and then avidin-conjugated fluoro-microbeads were injected. The bound fluoro-microbead conjugates were measured directly using a conventional fluorescence microscope. Fig. 3 shows the design and schematic diagram of the sandwich assay on fluoro-microbeads guiding chip.[53]
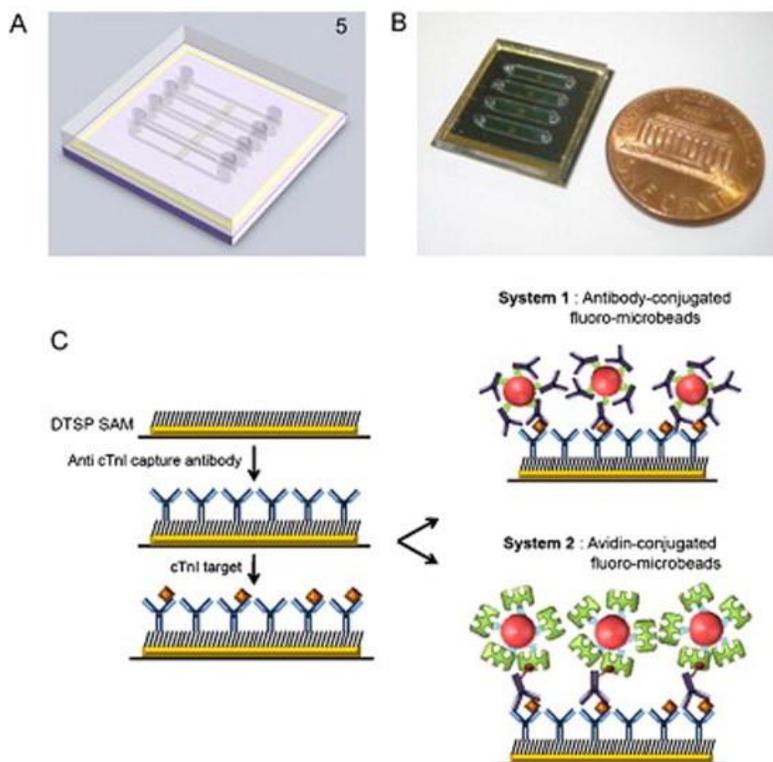

Fig.3. (A) Design of the fluoro-microbead guiding chip (FMGC). (B) Photograph of the FMGC. (C) Schematic diagram of the sandwich immunoassay using antigen–antibody binding (system 1) and avidin–biotin affinity binding (system 2) on the FMGC. The

avidin–biotin couple was used to enhance the signal.[53] DTSP, 3,3'-dithiobis-propionic acid *N*-hydroxysuccinimide ester; SAM, self-assembled monolayer.

Luminescence and colorimetric methods for cardiac troponin detection

Because of the need for 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.[54,55] For colorimetry assay, materials such as silicon chips,[56] glass surfaces,[57] and gold electrodes[58] have been developed. In 2010, Wu and coworkers proposed a biosensor based on the colorimetric method for cardiac troponin I detection. They showed that poly(dimethyl siloxane) (PDMS)–gold nanoparticles (AuNPs) composite film as a basis with silver enhancement can be employed by the colorimetric method for detection of cTnI.[59] PDMS has merits such as excellent transparency, outstanding elasticity, good thermal and oxidative stability, ease of fabrication, and the ability to be sealed with various materials. Gold nanoparticles can be functionalized as an idle substrate with antigen, enzyme, and other biomolecules. Hence, applying AuNPs patterned on PDMS films has a great advantage in biosensor applications. AuNPs aggregate in a liquid system; therefore, AuNPs are first immobilized onto PDMS film and then covered with antibodies. After blocking the surface with bovine serum albumin (BSA) and capturing cTnI, silver enhancement solution is dropped onto the surface of the sensor and live photos are taken. AuNPs play the role of catalyst during reactions of silver reduction, and when protein covers the surface of NPs, the catalytic property of AuNPs could be wasted. Differences in the type, quality, and quantity of proteins that cover the surface of AuNPs influence the amount of reduction in silver metal and cause the color differences of the reaction mixture. The detection of cTnI using PDMS–AuNPs composite film is practically used for clinical diagnosis. The experimental procedure and a schematic diagram of silver enhancement colorimetric detection of cardiac troponin I are shown in Fig. 4.[59]
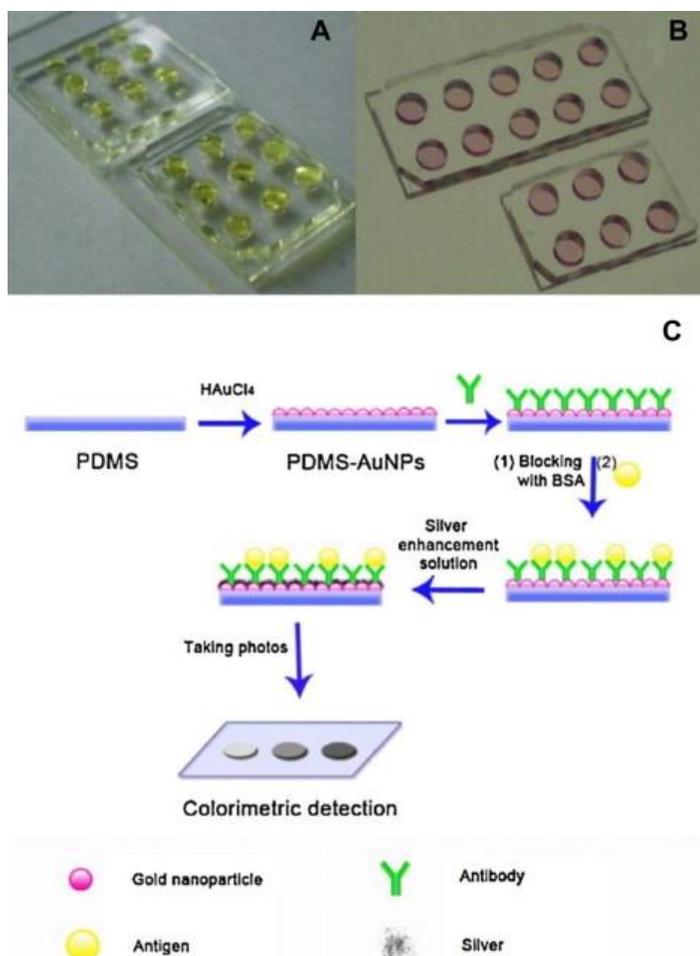
Fig.4. Experimental procedure for silver enhancement colorimetric detection of cardiac troponin I: (A) PDMS chip with HAuCl$_4$ solution; (B) photo of PDMS–AuNPs composite film; (C) schematic diagram for colorimetric detection.[59]

Chemiluminescence (CL) and electrochemiluminescence (ECL) biosensing platforms have been assessed for cardiac marker detection.[60,61] A novel nanoparticle based on an ECL immunosensor has been employed for sensitive detection of human cTnI.[22,62] The production of luminescence during electrochemical reaction is considered electrochemiluminescence. A high-sensitivity, wide-linear, low-background, and simple instrument that can be used for detecting the acute myocardial infarction biomarkers are taken 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.[63] AuNPs have been functionalized by protein via electrostatic and other physical adsorption mechanisms, and due to the high surface-to-volume ratio of AuNPs, biomolecules

can be immobilized to the surface of them. Gold nanoparticles were functionalized by using *N*-(aminobutyl)-*N*-(ethylisoluminol) (ABEI)– AuNPs and are used as labels via the simple seed growth method for ultrasensitive detection of cTnI in clinical diagnosis.[62] Two kinds of nanoparticles, SA–AuNPs (streptavidin-coated gold nanoparticles) and ABEI–AuNPs–Ab$_2$ nanoprobes, were used for the ECL immunosensor. SA–AuNPs enhance the conductivity or electron transfer between biomolecules and electrode and also improve immobilized capacity. There are two advantages to applying AuNPs in ABEI–AuNPs–Ab$_2$ nanoprobes. First, AuNPs carry antibodies for recognition of biomarkers. Second, numerous ECL signal-generating molecules of ABEI were immobilized on the surface of them for signal amplification. The biotinylated Ab$_1$ immobilized on the surface of SA–AuNPs/1,3-propanedithiol/Au, antigen cTnI, and ABEI–AuNPs–Ab$_2$ nanoprobes fabricate a sandwich-type ECL immunosensor. The design and fabrication process are shown in Fig. 5.
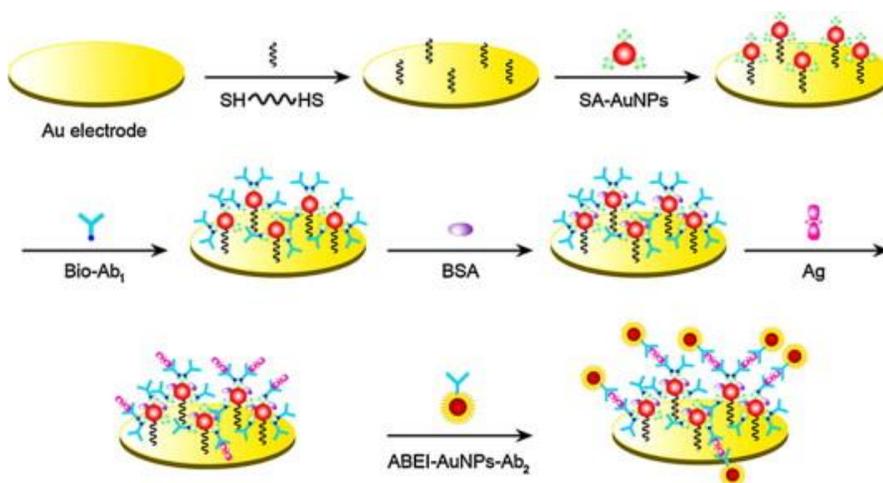

Fig.5. Schematic illustration of the fabrication process of the sandwich-type ECL immunosensor using SA–AuNPs as immobilization matrix and ABEI–AuNPs–Ab$_2$ as probes.[62]

According to Fig. 5, when the ABEI–AuNPs–Ab$_2$ probes were reacted with Ag/BSA/bio-Ab$_1$/SA–AuNPs/1,3-propanedithiol/Au substrate, strong ECL signals were obtained, and this is attributed to the ABEI–AuNPs–Ab$_2$ molecules attached on the electrode surface and is the result of the reaction of ABEI radicals electro-oxidized by ABEI with hydrogen peroxide (H$_2$O$_2$) besides the catalysis of AuNPs.

In 2013, Li and coworkers indicated the possibility of a sensitive label-free ECL immunosensor employed by using luminol-functionalized gold nanoparticles (luminol–AuNPs) as antibody carriers for detection of the cTnI acute biomarker.[22] Luminol–AuNPs conjugated biotinylated antibodies against cTnI (biotin–anti-cTnI–luminol–AuNPs) were assembled onto a streptavidin-coated AuNPs (SA–AuNPs) modified electrode.

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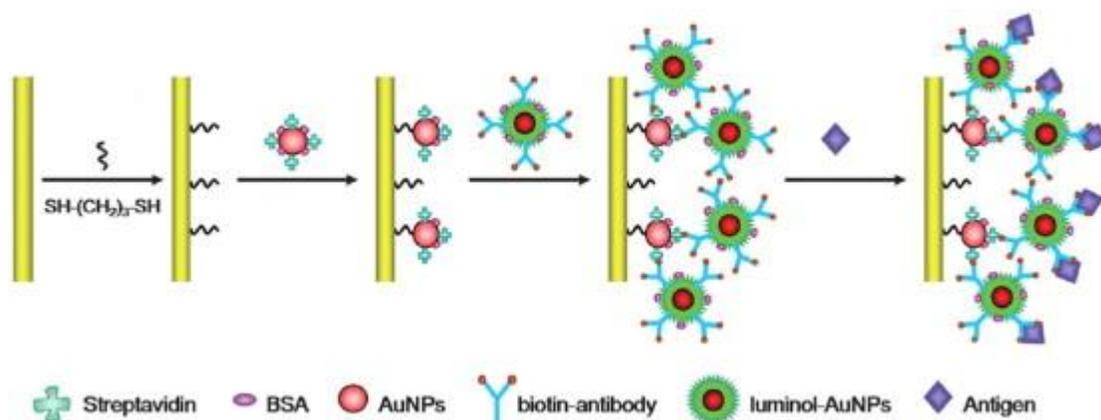

Fig.6. Schematic illustration of the fabrication processes of the label-free ECL immunosensor using luminol–AuNPs as antibody carriers and sensing platform.[22]

The ECL reaction of luminol with H_2O_2 formed electrotransfer interactions. In the presence of AuNPs as the catalyst and the absence of cTnI, luminal anions and HO_2^- in alkaline solution were electro-oxidized to $L_{\bullet}^-$ and $O_{2\bullet}^-$ radicals. In the presence of cTnI, biotin–anti-cTnI–luminol–AuNPs assembled Au electrode captured the target cTnI and an immune reaction happened. The ECL intensity is dependent on the concentration of cTnI.[22]

SPR-based biosensor for cardiac troponin detection

SPR immunosensing includes a thin metal film deposited on the surface of glass prism, and biorecognition elements containing proteins/antibodies/DNA/RNA have been immobilized on the surface of

the sensor.[64] When antigen reacts with the biorecognition element, the refractive index as a variation in light intensity changes.[65]

The surface plasmon resonance technique has been used for analytical application in immunosensors.[66] The high sensitivity to the variation of mass on the transducer surface in this technique results from the refractive index alteration by immobilizing antibodies in immunosensors.[48]

In 2007, Dutra and coworkers developed covalently immobilized antibody against cTnT on gold surface via a self-assembled monolayer (SAM) of thiols by using cysteamine coupling chemistry.[67] Immobilization methods are one of the most imperative aspects for the development of immunosensors.

SAMs have shown some advantages such as high stability, acceptable orientation, and easy preparation, and they became a method to immobilize biological elements for biosensor development. Mercapto groups attached rigidly versatile SAM to the gold electrode surface.[67] The amino groups of cysteamine SAM via cross-linking by glutaraldehyde immobilized antibodies on the surface of the sensor. A schematic of the SPR immunosensor for cTnT detection is shown in Fig. 7.
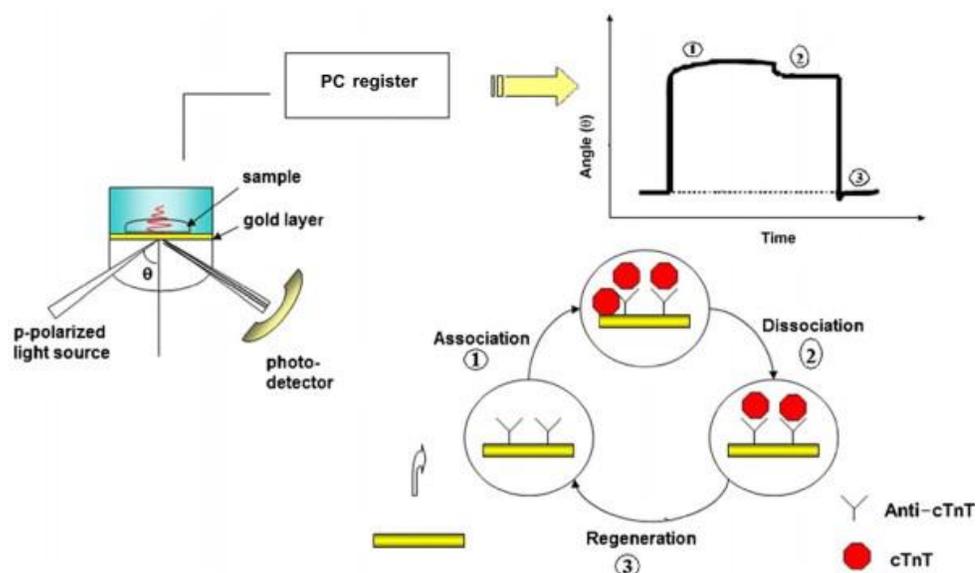

Fig.7. Schematic representation of the principle of SPR immunosensor for cTnT determinations.[67]

In this study with SPR software (version 4.1.2 from Eco Chemie), the response of the SPR sensor was automatically monitored and antibodies were immobilized covalently via the use of SAMs, and they allowed repetitive measurement with cost savings.[67]

In 2007, Dutra and Kubo proposed an SPR sensor in which streptavidin was immobilized on a replaceable carboxy methyl dextran hydrogel, which was used for binding biotinylated anti-troponin T monoclonal antibodies to detect human cardiac troponin T.[68] The SPR sensor component involved a gold-coated glass disc covered covalently with 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 a streptavidin-coated sensor disc and were monitored by observing changes in the resonant angle.[68] The interaction between biotinylated monoclonal antibodies for cTnT and streptavidin is shown in Fig. 8.

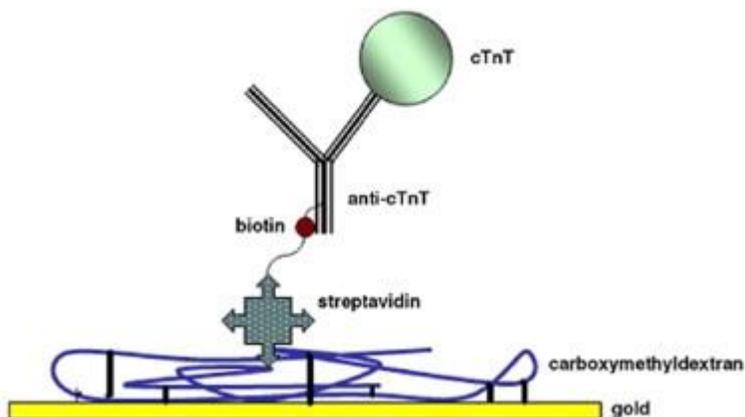**Fig.8.** Illustration of the immobilization scheme of biotinylated monoclonal antibody cTnT.[68]

The nonspecific binding on the surface is one of the major problems in this method, and it can make changes in the resonant angle. There are several ways to minimize this problem such as washing after the antigen recognition by immobilized antibody and blocking free reactive sites by a selective layer.[68]

A linear range of detection was from 0.03 to 6.5 ng/ml. The system presented was possible to measure the cTnT without dilution of the human serum with good specificity and reproducibility.[68]

In 2011, Liu and coworkers investigated immobilized troponin T antibody via SAM comprising a homogeneous mixture of oligo(ethylene glycol) (OEG)-terminated alkanethiolated and mercaptohexadecanoic acid (MHDA) on gold by using SPR. Oligo(ethylene glycol) resists the adsorption of protein from solution and has been employed for surface grafting. Because of high hydrophilicity, the hydrogen bond accepter, and the zero net charge, the SAM of OEG is suitable for making the antifouling surface. The functional group of the OEG cannot cross-link with biomolecules, and its antifouling ability is lost.[69]

For this reason, OEG was combined with MHDA and created a mixed SAM. The MHDA has carboxyl groups that can be coupled with amine groups on the antibody through 1-ethyl-3-(3-dimethylaminopropyl)carbodiimide hydrochloride (EDC)/*N*-hydroxysulfosuccinimide (sulfo-NHS) using active sites.[69]

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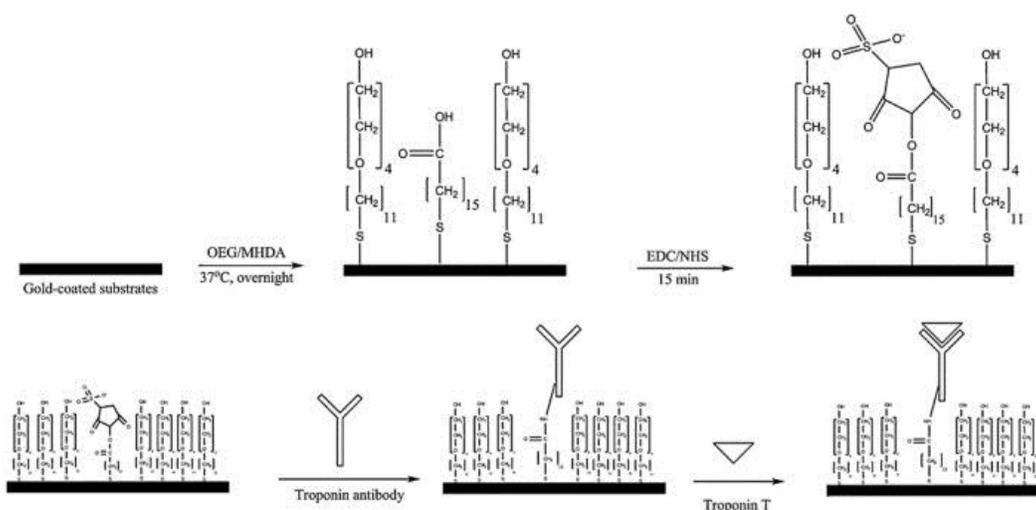

Fig.9. Schematic diagram of surface modification for the mixed SAM of OEG and MHDA and the immobilization method of antibody binding carboxyl groups of MHDA on Au.[69]

The SPR biosensor detected troponin T within 2 min after injection in a linear detection range lower than 50 μg ml^{-1}, and the limit of detection (LOD) of cardiac troponin T was 100 ng ml^{-1}, which is not yet sufficient for clinical diagnostics.[69]

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 a broad range of biological molecules, including proteins, small molecules, and nucleic acid.[70,71,72,73]

An optomagnetic biosensor based on nanoparticles that are magnetically actuated and optically detected in a sample fluid has been proposed for troponin detection.[74,75] There are electromagnets placed above and below the cartridge that generate magnetic forces, and supermagnetic particles are controlled and accelerated by them.[75]

In 2009, Bruls and coworkers coated the magnetic nanoparticles and the sensor surface with antibody, and magnetic nanoparticles were mixed into sample fluid in the cartridge.[75] Due to the large total surface area of the dispersed nanoparticles, nanoparticles captured analyte molecules.[76] Then, the electromagnets were applied to move the nanoparticles and localize them at the binding surface. For removing free and weakly bound particles from the surface, the magnetic field from the upper magnet is used. The optomagnetic system, with electromagnets and detection optics, was placed in the fluid microchamber.[75] The design and assay process steps are shown in Fig. 10.
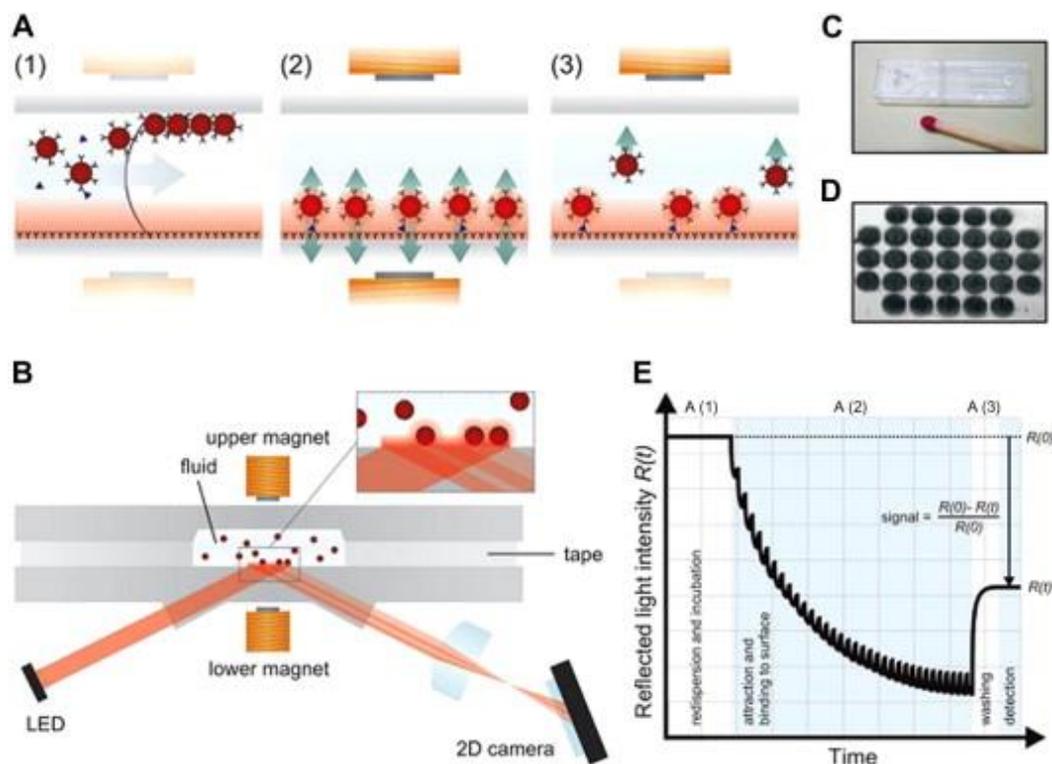
Fig.10. Optomagnetic immuno-biosensor based on actuated magnetic nanoparticles. (A) Schematic showing the assay process in the reaction microchamber: (1) antibody-conjugated magnetic nanoparticles fill microchamber and capture analyte; (2) electromagnets generate magnetic field that is applied on magnetic particles to binding to the surface; (3) magnetic field from upper magnet is used to remove unbound and weakly bound particles. (B) 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). 2D, two-dimensional. (C) Cartridge composed of two plastic parts connected by double-sided adhesive tape. The outer dimension of the cartridge is 1 × 4 cm. The cartridge includes 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 A(1) to A(3) are marked (see panel A). The signal modulation in phase A(2) is due to switched actuation of the magnetic nanoparticles.[75]

For detecting the presence of magnetic nanoparticles on the binding surface, the optical principle of frustrated total internal reflection (f-TIR)[77] was employed and is shown in Fig. 10B.[75]

Bruls and coworkers developed a fast and sensitive cTnI test based on actuated magnetic nanoparticles. For this purpose, nanoparticles that were incubated with sample fluid were injected into

the cartridge. The actuation protocol was started, and magnetic force from the lower magnet was applied to particles. Particles carrying cTnI were concentrated at the surface, and cTnI was bound between antibody-coated nanoparticles and antibody-coated surface of the sensor. Then, the optical signal was changed.[75]

Fluorescence resonance energy transfer (FRET) is known as a chemical transduction method that describes 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).[78]

In 2008, Stringer and coworkers employed biosensor-based FRET by using quantum dots as donors and organic dyes as acceptors for detection of troponin I.[79] To detect the fluorescence, a liquid core waveguide was used that was able to achieve highly sensitive and accurate measurement. For this purpose, green quantum dot was used as the donor with an emission peak wavelength at approximately 544 nm, and Invitrogen Alexa Fluor 546 (AF-546) fluorescent dye was used as acceptor, with the absorption peak wavelength directly overlapping with the emission peak wavelength of the donor. The amine-reactive ester on the surface of the quantum dot was created by using EDC and 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 include two F_{AB} arms and a single F_C stem, with F_{AB} being bound to a single F_C stem by means of a hinge region.[80,81] Protein A is simultaneously able to bind two IgG antibodies via the F_C portion of the IgG.[82] Quantum dot-labeled protein A and mouse anti-troponin I IgG monoclonal antibody-labeled AF-546 fluorescent dye were incubated for 2 h at room temperature. After that, protein A binds to anti-troponin antibody and self-assembled optical biosensor is produced. A schematic of the self-assembled optical biosensor architecture is shown in Fig. 11.[79]
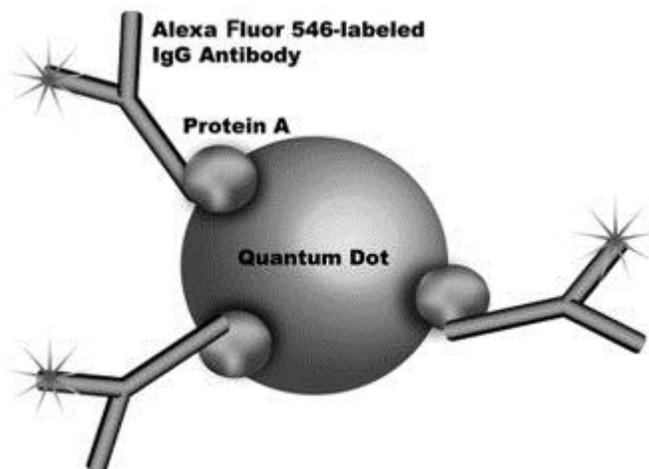
Fig.11. Schematic of self-assembled optical biosensor architecture.[79]

To obtain the emission spectrum with significant peak and large changes in energy transfer, the acceptor-to-donor ratio was given as 4:1 AF-546 to quantum dot. When antibody captures antigen, conformational change occurs within the antibody structure. Due to changing distances between quantum dot as donor and AF-546 as acceptor, the energy transfer is altered.[79]

Surface-enhanced Raman scattering (SERS) is a technique that offers orders of magnitude increases in Raman intensity, overcoming the traditional drawback of Raman scattering.[83] 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 occurs at a metal surface that has nanoscale roughness, and the molecules adsorbed onto that surface can undergo enhancement.[83,84] Typical metals used are gold and silver. Preparation of the surface can be provided through electrochemical roughening, metallic coating of a nanostructured substrate, or deposition of metallic nanoparticles (often in a colloidal form).

One of the more recently developed techniques for immunoassay and DNA detection is surface-enhanced Raman scattering.[85] This technique is of high interest due to its high sensitivity and potential for multiplexing.[62,86,87] Because Raman peaks are much narrower than fluorescence bands, SERS has potential for multiplex detection.[88] SERS assay was used recently via SERS nano-tags and magnetic beads for a sandwich immunoassay.[89,90] This method

includes two antibodies: one attached to the surface of magnetic beads and the other conjugated with a SERS nano-tag. As a result, the antigen is sandwiched between two antibodies.[91]

In 2014, Chon and coworkers developed a SERS-based competitive immunoassay for detection of cTnI and CK-MB.[91] For this reason, monoclonal antibodies for cTnI and CK-MB were immobilized onto the surface of magnetic beads, and two different types 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 immunocomplexes 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 used. Malachite green isothiocyanate (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.[91]

Conclusions and perspectives

The importance of cardiac troponin biomarkers for the fabrication of immunosensors and their application in acute myocardial infarction concept are known. Fabrication and modification of immunosensors by the 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 was summarized, and the conception of detection and development of devices based on optical detection principles was considered and implies that for fabrication of an ideal diagnosis of cardiovascular diseases at early stages, it is essential to detect cardiac troponin biomarkers with a 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. Several disadvantages of optical immunosensors, such as

bulkiness, high cost, and difficult labeling procedures, were described. Thus, clinical biosensors based on the colorimetric method using PDMS–AuNPs composite film with silver enhancement have been successfully attempted for cTnI detection in less than 20 min and a limit of detection of 0.01 ng/ml. Furthermore, the advantages of the colorimetric method have been considered in the ease of fabrication and operation and low cost, whereas in SPR immunosensors the detection of cTnI in a linear range from 0.03 to 6.5 ng/ml has been reported. In addition, the detection limit of the mentioned sensors was 0.01 ng/ml with the merits of good repeatability and specificity and concurrently compared to the fabrication strategy of the biosensor based on ECL immunosensors for the detection of cTnI. Moreover, ECL immunosensors, by using luminal–AuNPs as antibody with a wide range from 0.1 to 1000 ng/ml and a limit of detection of 0.06 ng/ml, have shown great application potential in clinical and pharmaceutical analysis, and the optomagnetic biosensor technology based on actuated nanoparticles for troponin detection has proposed advantages such as high-sensitivity, rapid multiplexed assays on a small sample volume and low-cost disposable cartridge.

Finally, the precision of troponin assay improvement will be continued and will ameliorate earlier diagnosis of acute myocardial infarction and better risk stratification on the use of the new highly sensitive assays. It is worth mentioning that testing for troponin has not been set at 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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Abbreviations used: AMI, acute myocardial infarction; CK-MB, creatine kinase MB; cTnI, cardiac troponin I; cTnT, cardiac troponin T; SPR, surface plasmon resonance; LSPR, localized surface plasmon resonance; NP, nanoparticle; PDMS, poly(dimethyl siloxane); AuNP, gold nanoparticle; BSA, bovine serum albumin; CL, chemiluminescence; ECL, electrochemiluminescence; ABEI, *N*-(aminobutyl)-*N*-(ethylisoluminol); SA, streptavidin; H_2O_2, hydrogen peroxide; SAM, self-assembled monolayer; OEG, oligo(ethylene glycol); MHDA, mercaptohexadecanoic acid; EDC, 1-ethyl-3-(3-dimethylaminopropyl)carbodiimide hydrochloride; sulfo-NHS, *N*-hydroxysulfosuccinimide; FRET, fluorescence resonance energy transfer; AF-546, Alexa Fluor 546; SERS, surface-enhanced Raman scattering.