CRITICAL REVIEWS IN ANALYTICAL CHEMISTRY
https://doi.org/10.1080/10408347.2022.2144995

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Development of Lateral Flow Assays for Rapid Detection of Troponin I: A Review

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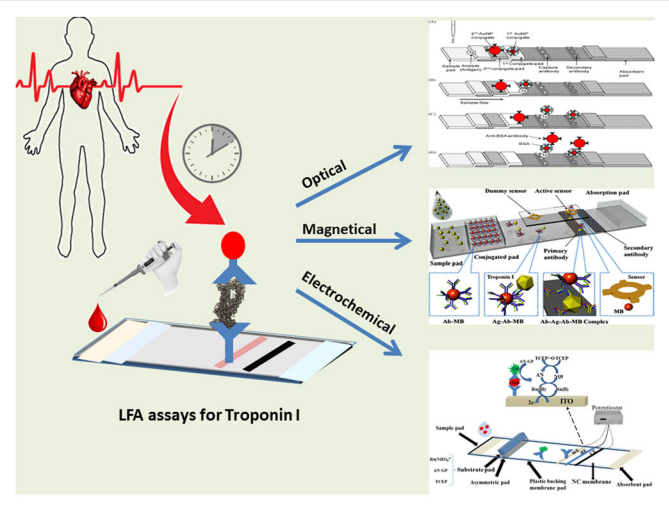
ABSTRACT

Troponin I as a particular and major biomarker of cardiac failure is released to blood demonstrating hurt of myocardial cells. Unfortunately, troponin I detection in the first hours of acute myocardial infarction usually faces with most negligence. Therefore, developments of point of care devices such as lateral flow strips are highly required for timely diagnosis and prognosis. Lateral flow assays are low-cost paper-based detection platforms relying on specific diagnostic agents such as aptamers and antibodies for a rapid, selective, quantitative and semi-quantitative detection of the analyte in a complex mixture. Moreover, lateral flow assay devices are portable, and their simplicity of use eliminates the need for experts or any complicated equipment to operate and interpret the test results. Additionally, by coupling the lateral flow assay technology with nanotechnology, for labeling and signal amplification, many breakthroughs in the field of diagnostics have been achieved. The present study reviews the use of lateral flow assays in early stage, quantitative, and sensitive detection of cardiac troponin I and mainly focuses on the structure of each type of developed lateral flow assays. Finally, this review summarized the improvements, detection time, and limit of detection of each study as well as the advantages and disadvantages.

KEYWORDS

Cardiac failures; lateral flow assay; troponin I

GRAPHICAL ABSTRACT



1. Introduction

According to World Health Organization (WHO), cardiovascular diseases (CVDs) annually take 17.9 million lives and are known as a major cause of death worldwide.[1] A serious and life-threatening condition of acute coronary syndromes (ACS) is acute myocardial infarction (AMI) which is a result of coronary arteries blockage. The lack of oxygen can result in permanent and irreversible damage to the heart

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muscle. Conventional, rapid and early AMI diagnosis is performed through electrocardiography (ECG) testing. Even though ECG is greatly specific for AMI, it has limited sensitivity. Not all patients that present with AMI have diagnostic ST-elevation ECG abnormalities. Thus, the ECG of AMI patients fall into two main categories: ST-segment elevation MI (STEMI) and non-ST-segment elevation MI (NSTEMI). Patients with a common chest pain manifestation must be evaluated for NSTEMI. ECG lacking any abnormalities or acute changes is prevalent in NSTEMI.[2]

Due to the low sensitivity of ECG for AMI diagnosis, troponins are commonly used for patients with chest pain that show no abnormalities in ECG. Cardiac troponin (cTn) is a cardiac regulatory protein that is responsible for regulating the calcium-mediated interactions between myosin and actin.[3] Troponin as a complex includes three units such as troponin I (cTnI), troponin T (cTnT), and troponin C (cTnC). Troponin is located on filaments of actin alongside tropomyosin proteins. Because cTnC is not cardiac-specific, it has no diagnostic value for cardiac injury assessment.[4] cTnT is the subunit that binds to tropomyosin, a regulator thin filament complex which converts calcium sensitivity to ATPase activity of striated muscle actomyosin. cTnI is a globular molecule with an MW of 22.5 kDa that binds to actin, cTnT, and cTnC. cTnI is the subunit that is responsible for muscle contraction inhibition in the absence of calcium. When Ca^{2+} is absent, in the presence of tropomyosin, cTnI inhibits the contractile interaction between actin and myosin.[5,6] Since cTnI is produced solely in the myocardium, it has a high specificity, thus assessment value for cardiac injury diagnosis,[7] where elevated levels of cTnI are an indicator of cardiac injuries.

Due to importance of patient-centered healthcare, early stage diagnosis and timely treatment, the Point of Care Testing (POCT) devices have gained a lot of attention.[8,9] Great attempts have been made to develop rapid, sensitive, user-friendly, on-site, and low-cost detection methods and devices. The conventional detection methods of analyte including enzyme-linked immunosorbent assay (ELISA) and real-time polymerase chain reaction (qPCR) have failed to provide fast results as well as offer a simple operation procedure. Recently paper-based devices have received considerable attention due to advantages of portability, cheapness, and environment-friendly.[10,11] Lateral flow assay (LFA), a paper-based detection system, is among the promising and leading diagnostic platforms for several advantages. LFA is simple to develop, affordable and more importantly, the test results are available within 5 to 30 minutes. Moreover, LFAs are versatile and can be utilized for detecting a broad range of analytes from antibodies to proteins, hormones, and even nucleic acids.[12,13] Lateral flow immunoassay (LFIA) is known as a paper-based assay in which antibodies have been employed as the recognition and detection component in the system.[14] A typical LFIA consists of sample pad, conjugate pad, detection zone, and absorbent pad. In the conjugate release pad, the detection complex consisting of anti-analyte antibody conjugated to the reporter particles (colored or fluorescent) are loaded. The sample migrates from the sample pad to the conjugate pad. Consequently, analytes interact with the detection complex and continue flowing down the strip, to the detection zone located on the nitrocellulose (NC) membrane. The detection zone consists of a test line (T-line), with secondary anti-analyte antibody, and the control line (C-line) that determines the proper sample flow down the test strip. The absorbent pad at the terminus of the test strip, ensures the flow from sample pad through the membrane as well as inhibiting the possible backflow in the system.[12] Sandwich type immunoassay, using two kinds of antibodies as capturing and detecting antibodies, is the major strategy that has been employed in LFIAs. In this system, the detecting antibody is responsible for signal generation. The generated signal may be electrochemical, optical, or magnetic signal.[15] As much as LFAs contributed to the fast, precise, and patient-centered diagnosis, their most important drawback such as qualitative feature may limit their usage. Conventional LFAs, which is commonly colorimetric-based, are not able to provide quantitative results for some clinical conditions which the exact amount of the target analyte is crucial for the diagnosis or patient's response to therapy. Microfluidic paper-based devices have gained a huge interest as powerful POCT devices which does not rely on yes or no results.[10,16] Microfluidic paper-based devices which are based on printed microfluidic patterns on the paper material, firstly were introduced in 2007 by Whitesides et al.[17] While electrochemical detection methods have been the top studied detection approach in microfluidic paper-based devices, fluorescent and chemiluminescent methods were also implemented for both quantitative and qualitative measurement.[18,19] Benefiting from microfluidic paper-based devices, Niu et al. developed an isoelectric focusing (IEF) device that enabled them to separate, isolate, and evaluate proteins in physiological samples.[20] Such attempts have been made to develop digitalized polymerase chain reaction (PCR) devices which quantitative and accurate DNA analysis was achieved by using only a smartphone as a POCT technique.[21–23] In order to enhancement of quantitative ability of LFA technique, application of nanoparticles with specific electrochemical, optical and magnetic features is highly demanded. Nanoparticles have been widely used in LFAs as labels for elevation of sensitivity of determination as well as the signal generation. In LFAs, nanoparticles are employed as labels of antibody or aptamer to capture and detect the target molecule and consequently, generate signals. Gold nanoparticles (AuNPs) are the mostly used label in optical detection-based LFAs. Other highly applied nanoparticle labels include quantum dots (QDs), silver nanoparticles (AgNPs), magnetic nanoparticles, selenium nanoparticles and carbon nanostructures.[24,25] In order to development and creating innovation in the structure of LFAs technique, different changes have been done in transducer systems to provide semi-quantitative and quantitative signals and enhance sensitivity. However, these changes may lead to expensiveness, time-consuming, and complexity of LFA technique. So in order to matching the LFA with POCT aims incorporation of

simplicity and miniaturization must be more consider in future studies.

Early and fast diagnosis of cardiac injuries by detecting specific cardiac biomarkers, especially in cases with no abnormalities in ECG, is crucial for treatment and preventing any permanent damages. Hence, in this paper, we reviewed studies that developed LFA platforms for the detection of cTnI, as a rapid, highly sensitive, and affordable alternative diagnostic device to conventional detection methods. The results of our review and details of studies are summarized in Table 1.

2. LFAs and optical detection mechanisms

2.1. Colorimetric detection

Among all the established LFAs readout mechanisms, optical readout methods are the most favorable. Specifically, color-based signal readouts are the most studied optical detection method for LFAs, since they offer simple detection and high sensitivity. The principle of measurement in colorimetric detection methods, is measurement of the optical density (OD), or contrast, of the developed color on the test line as a result of analyte's presence.[24,53] The most commonly explored colorimetric based LFAs are those with AuNPs as the label and color signal amplifier due to their distinctive optical characteristics.[54]

Two types of AuNPs, that are different in size, one for analyte detection and the other one for signal enhancement, were employed by Choi et al. As shown in Figure 1(A), the smaller AuNPs with the size of 10 nm diameter, were conjugated with anti-cTnI monoclonal antibody (mAb) 4T21, blocked by bovine serum albumin (BSA) and then were settled on the first conjugate pad. The bigger AuNPs with 40 nm in diameter, were conjugated with the anti-BSA antibody to bind to the smaller size AuNPs and were settled on the second conjugate pad. Capture mAb 19C7 and goat anti-mouse IgG antibody were used in the T-line and C-line on the nitrocellulose membrane, respectively. Signal amplification (color development) was obtained when the larger AuNPs-Ab was bound to the smaller AuNPs. The limit of detection for this method was obtained as 0.01 ng/mL with a detection time of 10 minutes.[26] A similar design using two types of AuNPs with different sizes (13 and 41 nm in diameter), for signal enhancement, was adopted in an LFIA for cTnI detection by Zhu et al. A biotinylated single-stranded DNA (ssDNA) was employed as a linker for two AuNPs. The 41 nm AuNPs that was labeled with streptavidin (first conjugate pad), was used as an amplifier for the 13 nm AuNPs (second conjugate pad) in order to magnify highly low signals. After sandwich formation between first anti-hs-cTnI antibody, and ssDNA/AuNPs (13 nm)-labeled second anti-hs-cTnI antibody on the T-line, enhancement was done through connection between streptavidin-labeled AuNPs (41 nm) and AuNPs (13 nm) by biotinylated ssDNA. The limit of detection for hs-cTnI by this method was 1 ng/mL.[27]

Elsewhere, LFAs based on a paper/soluble polymer hybrid were designed and developed by Han et al. which colorimetric detection was based on a smartphone. The developed LFA structure was designed by employing polyvinyl alcohol (PVA), a water-soluble and a fluid regulator polymer. The PVA barrier was fabricated and dispersed on the NC membrane of the test strips to control the fluids on the test strip. The design of lateral flow strip and detection process is shown in Figure 1(B). The standard production method was used to fabricate LFA strips. Anti-cTnI-conjugated AuNPs were employed as detection components of the LFA. Anti-cTnI capture antibody and anti-mouse IgG were used on the T-line and C-line, respectively. Prepared test strips in this study were consisted of absorbent pad, conjugate pad, NC membrane impregnated with PVA, sample pad, intermediate film, reagent pad, and buffer pad. The reagent pad contained the amplification reagent (Au ions) used for color signal amplification. The sample solution penetrated the sample pad and conjugate pad, and was blended with the AuNPs-Ab conjugates and afterward through the NC membrane, was transported to the absorbent pad for immunoreaction. At the same time, the reagent solution which was loaded on the buffer pad rehydrated the Au-ion reagent which was located on the reagent pad. Next, through the NC membrane, the mixture moved to the absorbent pad. The PVA barrier was gradually dissolved by the Au-ion reagent, while the immunoreaction was in progress. The Au-ion fluid (amplification fluid) was released to the NC membrane front as the result of the dissolving PVA barrier, and consequently signal amplification reaction was done at the test zone. Detection was done using the smartphone-based colorimetric reader and the results were obtained within 20 minutes, with a limit of detection of 0.92 pg/mL.[33]

Byzova et al. developed a colorimetric test strip for cTnI detection as well as fatty acid binding protein (FABP) and C-reactive protein (CRP). In this method, they conjugated AuNPs with anti-cTnI mAb C4 and used mAb C19 and goat anti-mouse IgG antibody on NC membrane for T-line and C-line, respectively. The detection time for this method was 10 minutes with a limit of detection of 1 ng/mL.[28] In another study, LFIA strips based on superspherical gold nanoparticles (S-AuNPs) were designed and fabricated in comparison with the usual quasispherical gold nanoparticles (C-AuNPs) for cTnI detection.[31] C-AuNPs were generated by the Turkevich–Frens method which is known as the citrate method. The obtained AuNPs by this method were spherical with a diameter between 10 to 30 nm.[55] However, the S-AuNPs were prepared using a progressive overgrowth technique. AuNPs were synthesized by seed-mediated growth method in a cetyltrimethylammonium bromide solution. The obtained S-AuNPs possessed optical as well as colloidal stability for a broad spectrum of sizes.[56] Both S-AuNPs (64.5 nm) and C-AuNPs (33.7 nm) were conjugated with IC4 mAb. Also mAb C19 and goat anti-mouse IgG antibodies were coated on the test zone and control zone, respectively. The S-AuNPs presented a limit of detection of 1.2 ng/mL for cTnI, while the limit of detection for C-AuNPs LFIA was reported 9.9 ng/mL. The better LOD for cTnI by S-AuNPs was due to the fact that these particles

Table 1. Summarized data of developed LFIA systems for detection of TnI.

Sensing method	Nanoparticle	Structure	LOD	Linear range	Detection (test) time	Ref.
Colorimetric	AuNPs	Ab and BSA conjugated AuNPs Anti-BSA antibody AuNPs	0.01 ng/mL	0.10–14.27 ng/mL	10 min	[26]
	AuNPs	Ab conjugated AuNPs Biotin-ssDNA-AuNP as amplifier	1 ng/L	1–10,000 ng/L	N.M.	[27]
	AuNPs	Ab conjugated AuNPs	1.0 ng/mL	3.0–100 ng/mL	10 min	[28]
	AuNPs	Ab conjugated AuNPs core–shell hybrid nanofibers	1 ng/mL	N.M.	10 min	[29]
	MB	Magnetic beads conjugated with Ab	0.1 ng/ml	N.M.	15 min	[30]
	C-GNPs S-GNPs	Ab-C-GNPs Ab-S-GNPs	9.9 ng/mL (C-GNPs) 1.2 ng/mL (S-GNPs)	N.M.	N.M.	[31]
	AuNPs MB	Ab-AuNP-MB	0.1 ng/mL	N.M.	20 min	[32]
	AuNPs	PVA dispersed on NC membrane, Ab conjugated AuNPs which amplified with gold ions	0.92 pg/mL	N.M.	20 min	[33]
Fluorescence	MS	Nile red wrapped MS conjugated with Ab	0.016 ng/mL	0–40 ng/mL	<15 min	[34]
	MS	Ab-protein G-MS	0.032 ng/mL	0.05–32 ng/ml	15 min	[35]
	Nanosphere	Ab-Cy5-cSA-NHS-PEG4-Biotin-BSA coated nanospheres	0.049 ng/mL	0.049–50 ng/mL	15 min	[36]
	GNR and Raspberry-type particle (a microparticle covered with europium chelate-contained silica nanoparticles)	Ab-GNR and raspberry-type microparticle conjugated with cTnI protein	97 pg/mL	0–1.16 ng/mL	N.M.	[37]
	CdSe/ZnS QD	Water-soluble silica-encapsulated QDs (QDs@SiO_2)	5.6×10^{-3} ng/mL	0.8–200 ng/mL	10 min	[38]
	–	Alexafluor 647-labeled Ab	0.019 ng/ml	0–100 ng/ml	<10 min	[39]
	CNF	Cellulose layered with CNF for signal amplification of F647-labeled Ab	1.28–1.40 ng/mL	Non-linear behavior	15 min	[40]
	ZrMOF@CdTe nanoparticles (NPs)	Ab-ZrMOF@CdTe	1 μg/L	N.M.	8 min	[41]
SERS	Rattle-like Au core in Ag-Au shell NPs (Au@Ag-Au NPs)	Ab-(Au@Ag-Au NPs)	0.09 ng/mL	0.09–50 ng/mL	15 min	[42]
	Gold nanorads	Au nanoprobes were taged with 1,4-nitrobenzenthiole (NBT) as Raman molecule were located in 1 nm gap between the Au nanorad core and Au shell.	0.1 ng/mL	0.1–0.3 ng/mL	N.M.	[43]
	AuNPs	Apt/silica/malachite green isothiocyanate/AuNPs	0.016 ng/ml	0.016–0.1 ng/ml	N.M.	[44]
Luminescence (Chemiluminescence, Electrochemiluminescence, Photoluminescence)	AuNPs	AuNPs-polyHRP-Ab conjugate	0.84 pg/mL	6 orders of magnitude	20 min	[45]
	AuNPs	AuNPs-(ald)HRP-Ab	5.6 pg/mL	5 orders of magnitude	25 min	[46]
	Mesoporous Silica Nanoparticles	Mesoporous silica nanoparticles (MSNs) loaded with tris(2,2′-bipyridyl)ruthenium (II) ($Ru(bpy)_3^{2+}$)	0.81 pg/mL	0.001–100 ng/mL	20 min	[47]
	Upconverting nanoparticle (UCNP)	Ab-UCNP	30 ng/L	30–10,000 ng/L	30 min	[48]
Magnetic-based	Superparamagnetic nanobeads (SPMNBs)	Ab coupled with SPMNBs	0.01 ng/ml	5 orders of magnitude	<15 min	[49]
	SPMNBs	Ab coupled with MB	0.42 nM	0.44–2.2 nM	1 min	[50]
	Magnetic beads (MB)	Ab-protein G-MB	0.01 ng/mL	0.01 − 0.1 ng/mL	N.M.	[51]
Electrochemical	–	Enzymatic effect of Gal-Ab for 4-amino-1-naphthyl b-D-galactopyranoside which triggers the RedOx cyclic of tris(3-carboxyethyl)phosphine, naphthoquinone imine, and $Ru(NH_3)_6^{3+}$ on ITO electrode	0.1 pg/mL	0.1 pg/mL to 100 ng/mL	11 min	[52]

N.M.: Not mentioned; SPMNBs: superparamagnetic nanobeads; MB: Magnetic beads; AuNPs: gold nanoparticles; C-GNPs: quasispherical gold nanoparticles; S-GNPs: superspherical gold nanoparticles; MS: polystyrene microspheres; GNR: Gold nanorod; QDs: quantum dots; UCNPs: upconverting nanoparticles; CNF: carbon nanofibers; Gal: β-galactosidase; HRP: horseradish peroxidase.

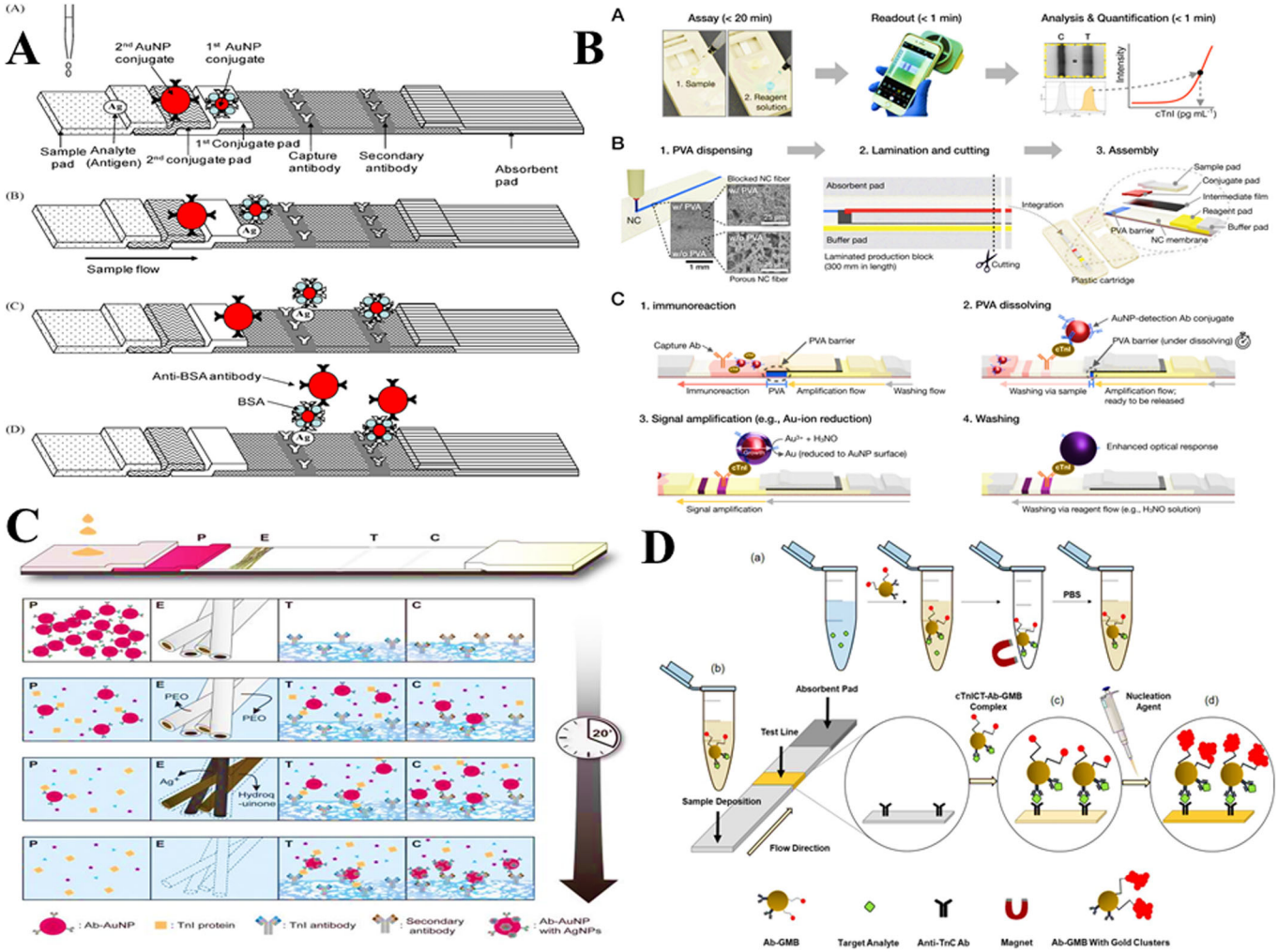

Figure 1. Schematic illustration of colorimetric LFA for TnI using signal enhancement step: (A) Larger AuNP-Ab binding to the smaller AuNP. ("Reprinted from Biosensors and Bioelectronics, Vol 25, Choi et al., A dual gold nanoparticle conjugate-based lateral flow assay (LFA) method for the analysis of troponin I, pp 1999–2002, Copyright 2010, with permission from Elsevier")[26]; (B) Au-ion fluid release by PVA dissolving in paper/PVA-based LFA. ("Reprinted with permission from,[33] Copyright 2020 American Chemical Society."); (C) Release of silver-enhancement reagents ($AgNO_3$ and hydroquinone) by dissolution water-soluble hybrid nanofibers on the NC membrane. ("Reprinted from Sensors and Actuators B: Chemical, Vol 273, Kim et al., Enhanced sensitivity of lateral flow immunoassays by using water-soluble nanofibers and silver-enhancement reactions, pp. 1323–11327, Copyright 2018, with permission from Elsevier")[29]; (D) Reduction of $HAuCl_4$ on MB-AuNPs (GMBs) on T-line. ("Reprinted from Sensors and Actuators B: Chemical, Vol 312, Sharma et al., Gold nanoparticle conjugated magnetic beads for extraction and nucleation based signal amplification in lateral flow assaying, p. 127959, Copyright 2020, with permission from Elsevier").[32]

with the size of 64.5 nm, provided a larger surface for Ab immobilization compared to 33.7 nm C-AuNPs. Another important factor which contributed to the improved detection of cTnI using S-AuNPs is that antibodies were efficiently immobilized on the surface of S-AuNPs without causing any variations in the curvature.[31]

Benefiting from silver properties for better color change for naked eye, Kim et al. designed a LFIA for detection of cTnI. In test strips, water-soluble hybrid nanofibers that contain silver-enhancement reagents ($AgNO_3$ and hydroquinone) were placed on the NC membrane, between the conjugate pad and the T-line on a LFIA strip (Figure 1C). The color change was induced as a result of binding of cTnI/Ab-AuNP complex to the capture antibodies on the T-line. By dissolution of the PEO shell due to the capillary flow, the reagents encapsulated in the hybrid nanofibers escaped. Consequently, silver ions were catalytically reduced on the captured AuNPs in the T-line. It is noteworthy that AuNPs' size was increased after reacting with the silver-enhancement reagents. This method exhibited a limit of detection of 1 ng/mL and detection time of 10 minutes with naked eye.[29]

A colorimetric detection assay was developed by Sharma et al. for detecting the cardiac troponin complex. In this strategy, the polystyrene magnetic beads (MBs) were bound with anti-troponin I antibodies, while the capture antibody on the T-line was an anti-troponin C antibody. An external magnetic field was employed under the sample zone to retain the MBs and preconcentrate the analyte (cardiac troponin complex). Later, by removing the external magnetic field, the analyte-MB-Ab conjugates flow down the membrane and subsequently were captured by anti-troponin C antibodies on the test zone. The formation of the Ab/analyte-Ab-MB sandwich complex led to brown color due to accumulation of MBs on the T-line. The protein A-conjugated MBs (PMBs) were employed for signal enhancement.

Protein A is derived from *Staphylococcus aureus* which is able to bind to the Fc segment of antibodies.[57] Thus, the brown color would be enhanced by binding PMBs to antibodies on the test zone. Thus, the sensitivity of the test would be increased. The visual detection limit and detection time for this method were reported to be 0.1 ng/mL and 15 minutes, respectively.[30] In a similar study, benefiting from MBs, AuNPs-conjugated MBs were used for signal amplification and improving the limit of detection. MB-AuNPs (GMBs) conjugates were labeled with anti-cTnI mAb via EDC/NHS method. For cardiac troponin complex detection, the anti-cTnC mAb was used as capture antibody on the T-line. Signal amplification is carried out by adding 5 µl of nucleation reagent on T-line and nucleation of gold nanocluster enhanced the contrast. The nucleation reagent for this strategy consisted of citrate buffer solution (pH = 4) including $NH_2OH.HCl$ and $HAuCl_4$. In the nucleation reaction, presence of AuNPs on the GMBs acted as seeds which in the presence of the reducing agent, the gold salt reduced on seeds. Consequently, gold clusters formed on GMBs, increased the optical signal, and the color contrast. Using this strategy, troponin complex was detected within 20 minutes with a limit of detection of 0.1 ng/mL.[32]

2.1.1. Critical note

The most frequently used nanoparticles, as label, for the reviewed LFIAs were AuNPs because they display optical characteristics which make them the ideal for colorimetric detection systems. Moreover, they are inexpensive to synthesize and the size of the particles can be readily controlled.[58] Although it has been demonstrated that AuNPs with particle size of 30 to 40 nm were the optimal choice for application in LFIAs,[59–61] it was observed that AuNPs up to 115 nm effectively contribute to decreasing the limit of detection.[62] Regarding these observations, the LFIA which employed superspherical AuNPs with average particle size between 20.2 to 64.5 nm displayed higher sensitivity toward cTnI and the limit of detection was significantly reduced compared to the LFIA using the usual AuNPs.[31] Besides AuNPs, magnetic nanoparticles were also used as labels in colorimetric detection since a brown color is developed as a result of MNPs' accumulation. Additionally, a bacterial antibody binding protein, protein A, was used for color signal amplification.[30] MNPs also can be applied for purification and isolation of the target analyte.[32] Various signal amplification methods were employed in LFIAs with a colorimetric detection method. A simple method for signal amplification was performed using binding of larger AuNPs-Ab to the smaller AuNPs on T-line.[26] An innovative approach for regulated and programmed signal amplification was achieved by encapsulating the reagent ($AgNO_3$ and hydroquinone) into carbon nanofibers for a gradual release as the fiber dissolved by the flow of the fluid.[29] In another LFIA device, using PVA barrier, the release of the Au-ions as signal amplification reagent was regulated.[33] Benefiting from the nucleation phenomenon, a citrate buffer solution containing $NH_2OH.HCl$ and $HAuCl_4$ was used leading to formation of gold particles on AuNPs seeds.[32]

2.2. Fluorescence detection

LFAs employing fluorescent signaling and labels which possess higher sensitivity as well as lower LODs. LFIAs based on fluorescent signaling can be categorized in three main groups. In the first group, the detection antibody is directly bonded to the fluorescent label. While in the other groups, the antibody is conjugated to either a package of fluorescent labels or an enzyme which is responsible for converting a quenched substrate to a fluorescent product.[15] Quantum dots (QD), fluorescent microspheres, and upconverting nanoparticles (UCNPs) are some of the mostly employed fluorescent labels in LFAs.[63]

By wrapping Nile red fluorescent dye inside microspheres, a fluorescence-based LFIA for cTnI detection was proposed by Cai et al.[34] Microspheres are nanostructures with several carboxyl groups on their surface which make them good candidates for coupling with antibodies via amide bond. The core-shell microsphere in this study was prepared using a one-step polymerization method. In this method, styrene was copolymerized with acrylic acid to generate polystyrene particles with surface carboxyl groups. This copolymerization was initiated by potassium peroxodisulfate (KPS) (Figure 2A) The Nile red-loaded microspheres were conjugated with anti-cTnI mAb 19C7 detection antibody. Two capture monoclonal antibodies of M18 and 560, were employed on T-line, and goat anti-mouse IgG antibody was used at C-line. The intensity of fluorescence emission was scanned, analyzed, and quantified by Feice Lateral Flow Reader. Using this LFIA, the results were obtained in less than 15 minutes and the cTnI levels were detected as low as 0.016 ng/mL. Another study demonstrated a fluorescent-based LFIA for cTnI detection by employing microspheres as detection nanoprobes. The microspheres were conjugated with anti-cTnI antibody 3H9 antibodies via Protein G. First, the Fc region of 3H9 was captured by the C1∼C3 domain of Protein G, then by using carbodiimide, the antibody was chemically bonded to Protein G. Finally, the Ab-Protein G complex was immobilized on the surface of microspheres. Alexa fluor 647 NHS ester (F647), a photostable far-red dye, was employed as the fluorescent agent in the fluorescent detection nanoprobes. To scan and analyze the fluorescent signals after the immunoreaction, fluorescent quantitative immunoassay analyzer was used. In this method, the detection results were obtained within 15 minutes and LOD for cTnI was 0.032 ng/mL.[35]

Moreover, Lou et al. designed a highly sensitive fluorescent-based LFIA by sequentially labeling detection nanospheres containing biotin, NHS-PEG4-biotin and core streptavidin (cSA) in which cSA acts as carrier for Cy5-NHS ester as the fluorescent label. Later, NHS-PEG4-biotin was attached on the cSA, and 3H9 detection antibody was conjugated on NHS-PEG4-biotin. The final detection nanoprobe contained a structure of Ab/Cy5/cSA/NHS-PEG4/Biotin/BSA-coated polystyrene nanospheres. This structure of LFIA enabled detection of cTnI with LOD of 0.049 ng/mL within 15 minutes.[36] In this way, Wu et al. generated a core-shell CdSe/ZnS QDs using a green phosphine free method. Highly dispersed aqueous SiO_2@CdSe/ZnS QDs were

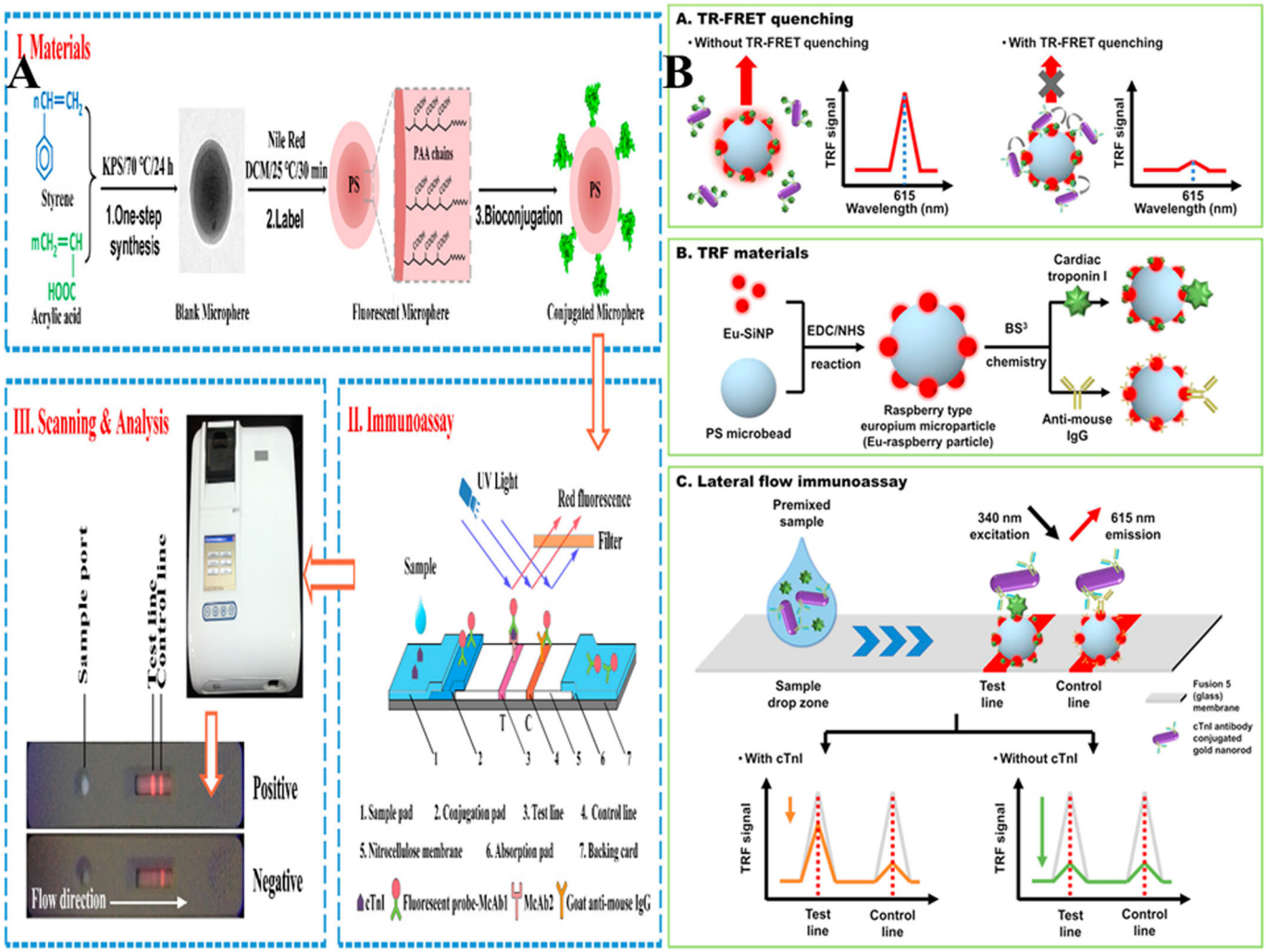

Figure 2. Schematic illustration of fluorescence LFA for cTnI using: (A) Nile red-loaded polystyrene microspheres. (Reprinted from Molecules, Vol 23, Page 1102, Figure 1 (https://www.mdpi.com/1420-3049/23/5/1102/htm), © 2018 by Cai et al., Licensee MDPI, Basel, Switzerland. This article is an open access article distributed under the terms and conditions of the Creative Commons Attribution (CC BY) license (http://creativecommons.org/licenses/by/4.0/))[34]; (B) TR-FRET quenching phenomenon between Eu-SiNPs and gold nanorads. (Reprinted from Biosensors and Bioelectronics, Vol 163, Lee et al., Time-resolved fluorescence resonance energy transfer-based lateral flow immunoassay using a raspberry-type europium particle and a single membrane for the detection of cardiac troponin I, p. 112284, Copyright 2020, with permission from Elsevier).[37]

prepared via a modified Stöber method. In comparison with bare QDs prepared by conventional method, the SiO_2@CdSe/ZnS QDs showed low cytotoxicity and better stability. The generated SiO_2@CdSe/ZnS QDs were bonded with anti-cTnI mAb1 and goat anti-rabbit IgG to serve as test probes and control probes, respectively. The probes were placed on the conjugate pad on the test strips. On the LFIA strips, the anti-cTnI mAb2 and anti-rabbit IgG antibody were printed on T-line and C-line, respectively. By adding the sample solution on the sample pad, the analyte flowed through the strip and was captured by test probes on the conjugate pad. Later, the cTnI-test probe conjugate was captured at the T-line and detection was carried out via fluorescent signal readout. The LOD for cTnI in 10 minutes was obtained as 5.6×10^{-3} ng/mL.[38]

To improve the sensitivity of the fluorescence-based LFIA, an LFIA by employing the time-resolved fluorescence resonance energy transfer (TR-FRET) quenching phenomenon was developed. Here, europium chelate-containing silica nanoparticles (Eu-SiNPs) and gold nanorods were used as fluorescence donor and fluorescence acceptor, respectively. Polystyrene microbeads were conjugated with Eu-SiNPs to obtain a raspberry-type europium microparticle (Eu-raspberry particle). Next, they were conjugated with either cTnI antigen or anti-mouse IgG antibody and were dispersed on the Fusion 5 membrane at T-line and C-line, respectively. Gold nanorods were bonded with anti-cTnI antibodies and were mixed with sample solutions and added to membrane (Figure 2B). In the presence of cTnI, when the cTnI-Ab-gold nanorods were captured by Eu-raspberry particle conjugated cTnI on the T-line, the fluorescence signal was slightly decreased on the T-line. However, when cTnI was absent, the signal dropped drastically as a result of TR-FRET quenching. The quantitative assessment of cTnI can be achieved by evaluation of the fluorescence signal between the Eu-raspberry particle and gold nanorods. This LFIA enabled detection of cTnI at concentrations as low as 97 pg/mL.[37]

In another study Natarajan et al. introduced a fluorescence-based LFAI, where the fluorescence intensity of the

cellulose membrane was enhanced by coating the membrane with carbon nanofibers at the detection zone. The carbon nanofibers enhanced the signal generation by increasing the number of capture antibodies on the surface of the strips. The mouse anti-cTnI 19C7 detection antibody was labeled with F647 and the capture antibody used on the T-line was mouse anti-cTnI 4C2 antibody. The results indicated that fluorescence signals generated as a result of the immunoreaction in the system, were similar to the results of the NC membrane-based LFIA for cTnI detection, and cellulose membranes can be considered as alternatives to NC in LFIA test strips. The limit of detection and the detection time of cTnI for this cellulose-based fluorescent LFIA was reported to be 1.28–1.40 ng/mL and 15 minutes, respectively.[40]

Zou et al. prepared a highly luminescent zirconium metal organic framework (ZrMOF) nanoparticles loaded with cadmium tellurium quantum dots (ZrMOF@CdTe) via a hydrothermal strategy. In these fluorescent-based LFIA strips, the conjugate pad was taken out since ZrMOF@CdTe QDs/anti-cTnI nanoparticles were directly mixed with sample solutions and added to the sample pad. The generated fluorescence of the immunoreaction at the T-line can be observed by naked eye under 365 nm ultraviolet light. The cTnI can be detected within 8 minutes even at concentrations as low as 1 μg/L.[41]

A photoluminescence-based LFIA was designed by Bayoumi et al. for quantitative and sensitive cTnI detection by incorporating upconverting nanoparticles (UCNPs) as detection probes. UCNPs display distinct photon upconversion luminescence by transforming low-energy excitation wavelength (near or at infrared) to high-energy emission at visible wavelengths. In this study, carboxylated UCNP-reporter particles with a hydrophilic coating were conjugated with anti-cTnI mAb 625. UCNP-Ab conjugates solution was mixed with sample solution and then added on the sample pad. After 30 minutes the mixture was absorbed to the test strips, and the results were read by LFIA reader which excites the UCNPs at 976 nm and the emission was measured at 540 nm. The LOD for cTnI was reported to be 30 ng/L.[48]

2.2.1. Critical note

In LFIAs which employed fluorescent means for detection, the fluorescent emitting source is either the fluorescent dye or the nanoparticles. In either method, the fluorescent signal is generated as the result of immunoreaction. Additionally, these types of LFIAs did not have signal amplification step. SiO_2@CdSe/ZnS QDs[38] and ZrMOF@CdTe QDs[41] were two types of novel synthesized nanoparticles in LFIAs for cTnI detection which demonstrated very fast (10 minutes and less) and sensitive cTnI detection. In LFIAs which used fluorescent dyes, like Nile red or F647, they were mainly conjugated on the detection nanospheres or microspheres.[34–36] In one LFIA, no nanoprobe was employed for detection and the F647 dye was directly conjugated to the anti-cTnI detection antibody. In this LFIA, the detection time was faster (10 minutes) compared to LFIAs employing dye conjugated nanoparticles.[39,40]

2.3. Luminescence and other optical detection methods

Luminescence-based biosensors and immunoassays have been rapidly developed and depending on the excitation method, luminescence-based assays can be categorized into photoluminescence, chemiluminescence, radioluminescence, thermoluminescence, triboluminescence, and sonoluminescence.[64] Chemiluminescence techniques are highly detectable and can be efficiently and easily measured. Another major advantage of luminescence-based analysis systems over fluorescent-based detection platforms, is inability of detection of low concentrations of the analyte by fluorescent-based biosensors due to interferences greatly enhanced by the photobleaching. Luminescence-based LFIAs are a growing field of study and have gained a great attraction due to simple luminescence detection principle as well as the convenient use of the paper-based devices. Various labels like horseradish peroxidase (HRP) have been implemented to improve the sensitivity and analytical performance of the chemiluminescence-based LFIAs.[65] Electrochemiluminescence is another technique used in luminescence-based immunoassay systems which unlike chemiluminescence, chemiluminescent reaction generating reactive species are produced electrochemically at the surface of an electrode, from precursors. One of the most commonly employed labels in electrochemiluminescence is a ruthenium, tris(bipyridyl) chelate, in which via an oxidation reduction–type reaction with tripropylamine, electrochemiluminescent signals are generated at an electrode.[66]

Han et al. developed a highly sensitive chemilumenescence LFIA strip using functionalization of AuNPs with HRP, and a streptavidin-polymer backbone conjugate followed by adding the biotynilated anti-cTnI antibodies to the conjugate, forming an AuNP-polyHRP-Ab complex (Figure 3A).[45] The amplification was carried out based on a water-swellable polymer which was composed of polyvinyl alcohol (Figure 3A).[67] The amplification site was prepared by layer-by-layer attaching laminating supporting film to adhesive film, swellable polymer, flow-control film, solution-containing pads and adhesive film. The swellable polymer expanded vertically when contacted with aqueous solutions. The vertical expansion acted as a fluid switch. When the immunoreaction proceeded on the test strip, as the polymer expanded, the amplification site moved toward the test strip. The amplification site which was bound to the test strip released the reagent solution (CL) of luminol-H_2O_2 at the considered time when the immunoreaction was saturated. Thus the test strip flow changes from an immunoreaction to signal amplifying reaction. The cTnI was detected within 20 minutes with a limit of detection of 0.84 pg/mL.

An electrochemiluminescence-based LFIA was introduced by Hong et al. which employed mesoporous silica nanoparticles (MSNs) embedded with tris(2,2′-bipyridyl)ruthenium (II) ($Ru(bpy)_3^{2+}$) (RMSN) for cTnI detection (Figure 3B). Due to its unique optical characteristics, great electrochemical stability, and water-solubility, $Ru(bpy)_3^{2+}$ is a commonly used photoactive species in sensing platforms that are based on electrochemiluminescence owing to its unique optical characteristics, great electrochemical stability, and water-solubility. Next, nanoparticles were conjugated with 4T21-

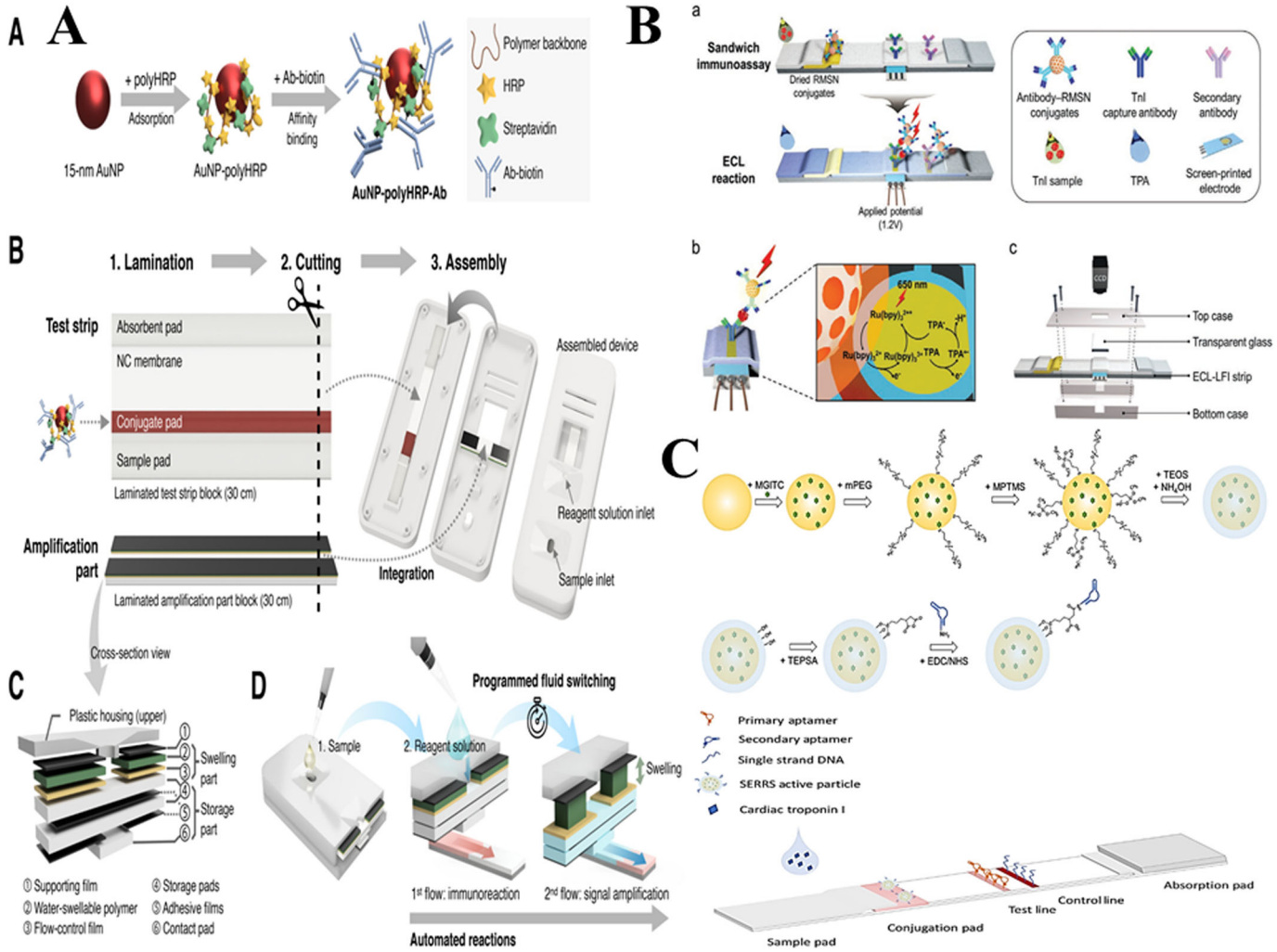

Figure 3. Schematic illustration of LFIA strip based on: (A) Chemiluminescence using AuNP-polyHRP-Ab. ("Reprinted with permission from,[45] Copyright 2019 American Chemical Society."); (B) Electrochemiluminescence using mesoporous silica nanoparticles loaded with $Ru(bpy)_3^{2+}$. (Reprinted from Small, Vol 16, Kim et al., Ru(bpy)32 -Loaded Mesoporous Silica Nanoparticles as Electrochemiluminescent Probes of a Lateral Flow Immunosensor for Highly Sensitive and Quantitative Detection of Troponin I, p. 2004535, Copyright 2020, With permission from John Wiley and Sons)[47]; (C) SERRS using aptamer/silica/MGITC/AuNPs. (Reprinted from Journal of biomedical optics, Vol 25, p. 097001, Figures 1 and 2 (https://doi.org/10.1117/1.JBO.25.9.097001), © Tu et al. Published by SPIE under a Creative Commons Attribution 4.0 Unported License (http://creativecommons.org/licenses/by/4.0/)).[44]

19C7 antibodies. 4T21-560 antibody was applied as capture at T-line and anti-mouse IgG antibody at C-line on NC membrane. Tripropylamine (TPA) as electrochemiluminescence signal generation reagent was added on the sample leading to the electrochemically reacting with $Ru(bpy)_3^{2+}$ on the T-line. Using this method, the detection time for cTnI was reported to be 20 minutes with a limit of detection of 0.81 pg/mL.[47]

Surface-enhanced Raman scattering (SERS)-based LFIAs have been developed for quantitative and highly sensitive detection. Except for the SERS probes, in SERS-based LFIAs, the primary principles as well as the configuration are in accordance with those of the conventional AuNPs-based LFAs. Bai et al. developed LFIAs based on SERS signaling for quantitative detection of cTnI, as well as the colorimetric detection. In this study, not only conventional citrate-capped AuNPs were used as detection probes, but also three types of Au-Ag bimetallic nanoparticles (Au-Ag NPs), rattle-like Au core in Ag-Au shell nanoparticles (Au@Ag-Au NPs), and Au core with Ag shell nanoparticles (Au@Ag NPs) were generated to benefit from excellent SERS activity of Ag nanoparticles. Moreover, the beforementioned particles were tagged with Nile blue A (NBA) as a SERS tag in SERS-based quantitative detection. All four types of probes were conjugated with anti-cTnI mAb 16A11 and mAb 19C7 and goat anti-mouse IgG were employed on NC membrane in T-line and C-line, respectively. According to the results of this study, Au@Ag-Au NPs exhibited the strongest SERS activity and accordingly, the most sensitive cTnI detection was achieved within 15 minutes with an LOD of 0.09 ng/mL, in test strips incorporating detection probes of Au@Ag-Au NPs.[42] Another SERS-based LFIA for cTnI detection was developed by Khlebtsov et al. which employed an Au nanoprobe consisting of Au nanorod core and Au shell with a 1 nm gap between the core and shell. Au nanoprobes were tagged with 1,4-nitrobenzenthiole (NBT) as Raman molecule. NBT tag was placed in the 1 nm gap space of the Au probe, between the core and shell. Finally, the nanoprobes were bonded with anti-cTnI IC4 antibody. This SERS-based LFIA showed an LOD of 0.1 ng/mL for cTnI.[43]

Tu et al. developed an LFA based on aptamers developed by surface-enhanced resonance Raman spectroscopy (SERRS) signaling for cTnI detection. In this detection platform, aptamers were employed as a substitute to antibodies. Aptamers are short, single-stranded oligonucleotides (ssDNA or ssRNA) with high selectivity and affinity toward their targets. Hence, they have gained a great attention as detection probes in biosensor platforms.[68,69] In this study, a biotinylated capture aptamer (primary aptamer) with sequence of 5′-biotin-TTTTTTCGTGCAGTACGCCAAC CTTTCTCATGCGCTGCCCCTCTTA-3′ was used on the T-line while the detection aptamer for cTnI with the sequence of 5′-amine-spacer 18-spacer 18-CGCATGCCAAACGTTG CCTCATAGTTCCCTCCCCGTGTCC-3′,[70,71] was immobilized on silica-coated AuNPs previously conjugated with malachite green isothiocyanate (MGITC), as a Raman reporter (Figure 3C). The control DNA strand at the C-line on the NC membrane was 5′-biotin-TTTTTTGGACA CGGGGAGGGAACTATGAGGCAACGTTTGGCATGCG-3′. To collect and measure the SERRS signals on T-line, a portable Raman spectrometer was used. This new aptamer-based detection platform exhibited remarkable affinity and selectivity toward cTnI and an LOD of 0.016 ng/ml was reported.[44]

2.3.1. Critical note

Core-shell nanoprobes were highly employed for SERS signaling and Raman molecules like NBT or NBA were mostly resided in the gaps between the core and the shell of the particles such as core-shell Au@Ag-Au NPs[42] and core-shell AuNPs.[43] Moreover, both Ag and Au nanoparticles have good SERS activity.[72] Almost all LFA devices used for cTnI detection, relied on antibodies to detect cTnI in the given samples. However, in one study, cTnI specific aptamers were employed instead of antibodies. Aptamers are short oligonucleotides, and they display great selectivity and specificity toward their target. Moreover, they are inexpensive and easy to synthesize compared to antibodies. The silica encapsulated MGITC-conjugated AuNPs were functionalized with cTnI-specific aptamers and the SERS signaling was used for quantitative measurement of the cTnI. Aptamer-based LFA showed a great sensitivity toward cTnI as other LFIAs.[44]

3. Magnetic-based detection

Magnetic nanobeads (MNBs) are key components for the development of new generation of LFIAs for sensitive and quantitative detection of the desired analyte. MNBs could be employed for quantitative measurements with an external reader. In LFAs, MNBs have to be functionalized with an active biomolecule that recognizes specifically the analyte of interest.[73] In LFIAs, bio-conjugates of MNB-Ab are applied for a quantitative and highly sensitive detection of the analyte. MNBs are mostly composed of magnetite (Fe_3O_4) and maghemite (γ-Fe_2O_3) which are metallic materials. Additionally, they are superparamagnetic when their size is below 20 nm. They exhibit paramagnetic characteristics when they are about 30 to 100 nm in size.[74]

Benefiting from advantages of superparamagnetic nanobeads (SPMNBs) for cTnI detection, Xu et al. employed SPMNB-Ab which were obtained using troponin I monoclonal antibody, mAb1-16A11, and were later employed on the conjugate pad. For the T-line and C-line on the NC, anti-human cTnI mAb2-19C7 and goat anti-mouse IgG were employed, respectively. 30 minutes after applying the sample on the sample pad, for detecting the magnetic signal of SPMNB, test strips were inserted in the Magnetic Assay Reader. The total number of SPMNBs that was evaluated by the Magnetic Assay Reader in the T-line could indicate the related amount of cTnI in the sample. Hence, the more mAb2-cTnI-mAb1-SPMNB complexes were caught and formed in the T-line, the stronger the magnetic signal of the T-line will be detected. This can be indicated as a greater amount of cTnI in the sample. The detection time for this test was less than 15 minutes with an LOD of 0.01 ng/mL.[49]

In other study by Ryu et al., for increase the sensitivity of the LFA, the magnetic beads (MBs) bonded with protein G via EDC/sulfo-NHS coupling reaction. Protein G is a cell wall protein with immunoglobulin and serum albumin binding domains on its extracellular domain.[75] The immunoglobulin binding domain of Protein G only binds to the Fc domain of the antibodies. The MB-protein G conjugates were modified by mAb1 (16A11). As for the test strips, 19C7 monoclonal antibodies were employed on NC in T-line. The detection was carried out using Giant magnetoresistance (GMR) sensor. This purposeful immobilization of antibodies on MBs increased the sensitivity of the assay, and the developed LFA for cTnI exhibited a limit of detection of 0.01 ng/mL.[51]

Elsewhere, the SPMNBs-mAb1 (16A11) was used as detection platform for cTnI. On the test strips, anti-human cTnI mAb-19C7 and goat anti-mouse IgG were employed on T-line and C-line, respectively. However, in this study, the detection of cTnI was carried out using a highly sensitive hybrid Anisotropic Magnetoresistance/Planar Hall Resistance (AMR/PHR) ring sensor (Figure 4).[50] The noble fabricated ring sensor functioned like a Wheatstone bridge type sensor array. The sensor was fabricated using a bilayer magnetic thin film layered by a DC magnetron sputtering system.[76] When the mAb2-cTnI-mAb1-SPMNB complexes were formed in the T-line, the sensor was mechanically located on the test zone for signal detection. The voltage variation of the sensor is relative to accumulation of the SPMNBs in the T-line. This method was able to detect and quantify cTnI under 1 minute with the limit of detection range of 0.44–2.2 nM.

3.1. Critical note

In magnetic-based LFIAs, the nanoparticles were superparamagnetic nanobeads (SPMNBs)[49,50] and magnetic nanobeads (MBs)[51] which were functionalized with anti-cTnI antibody. In the study by Ryu et al., the MBs were first conjugated with protein G, an immunoglobulin binding protein,

and then were modified with anti-cTnI antibodies to ensure the maximum cTnI capture by the Fab region of the antibody since protein G only binds to the Fc region of immunoglobulin. Hence, the sensitivity of the LFIA increases.[51] The magnetic signals are detected by sensors and the signals are proportional to the cTnI concentrations, enabling quantitative measurement of the analyte. In another study, a novel highly sensitive hybrid Anisotropic Magnetoresistance/Planar Hall Resistance (AMR/PHR) ring sensor was developed which enabled the real-time displaying of the sensor voltage in various concentrations of cTnI and displayed a two-step increments with time. The change of voltage in early stage was heavily influenced by the kinetics of immunoreaction. Then, the change of steady stage was controlled by Ab-cTnI complex mass-transport kinetics.[50]

4. Electrochemical detection

An interference-free electrochemical LFIA was introduced by Akanda et al. using the electrochemical–chemical–chemical (ECC) redox cycling of 4-amino-1-naphthol (AN) combined with an enzymatic reaction. ECC redox cycling included an enzyme label for signal amplification, enzyme product, $Ru(NH3)_6^{3+}$ as catalytic agent, and tris(3-carboxyethyl)phosphine (TCEP) and β-galactosidase (Gal) as a label for signal amplification. As shown in Figure 5, the test strip consisted of a sample pad, substrate pad, asymmetric polysulfone membrane, NC membrane, indium–tin oxide (ITO) electrodes, and absorbent pad. On the test strips, anti-cTnI capture Ab was dispersed on the NC membrane near the ITO electrode and the detection Gal-conjugated Ab was placed 1 cm apart from the capture Ab, between the substrate pad and the capture Ab. On the substrate pad, $Ru(NH3)_6^{3+}$, TCEP, and 4-amino-1-naphthyl b-D-galactopyranoside (AN-GP) were dried. The asymmetric pad which possessed asymmetric pore sizes, where size gently increases from one side to the other, was placed under substrate pad. The substrate pad and the symmetric pad were first assembled together and then were assembled on the test strips in the way that smaller pore size surface of the asymmetric membrane was in contact with the NC membrane. As a result of inadequate capillary action from the smaller pore to the larger pore, the asymmetric membrane pad was gradually vertically wetted. Consequently, the substrates in the substrate pad were dissolved and distributed on the NC membrane. During the flow, troponin I bonded with Gal-conjugate Ab and then to capture Ab. The asymmetric membrane pad was gradually soaked, resulted in release of AN-GP, $Ru(NH3)_6^{3+}$, and TCEP on the substrate pad with a time delay and then transferring to the absorbent pad.

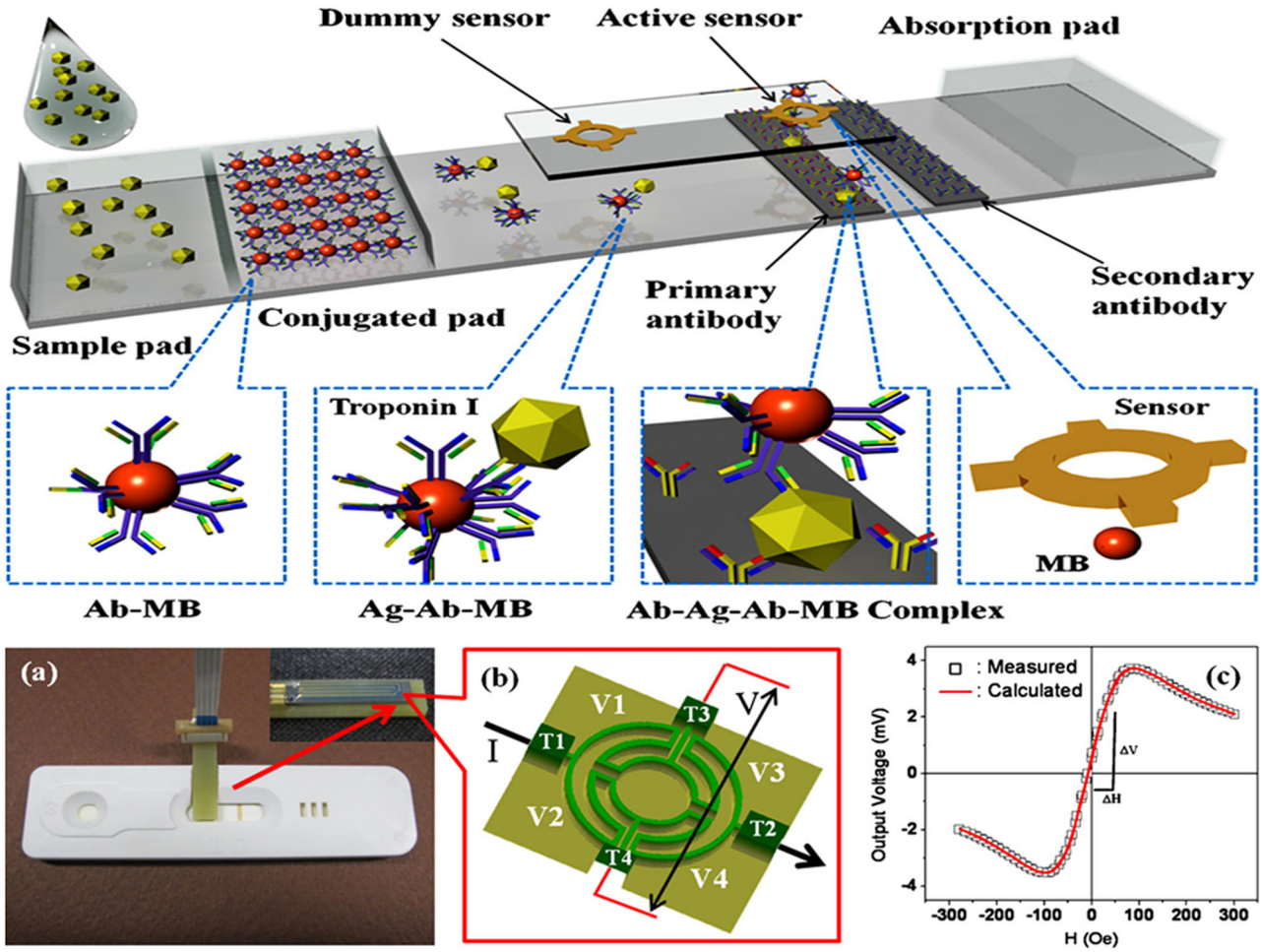

Figure 4. Schematic illustration of magnetic based LFA using hybrid AMR/PHR ring sensor. ("Reprinted from Sensors and Actuators B: Chemical, Oh et al., Vol 160, Analytes kinetics in lateral flow membrane analyzed by cTnI monitoring using magnetic method, pp. 747–752, Copyright 2011, with permission from Elsevier").[50]

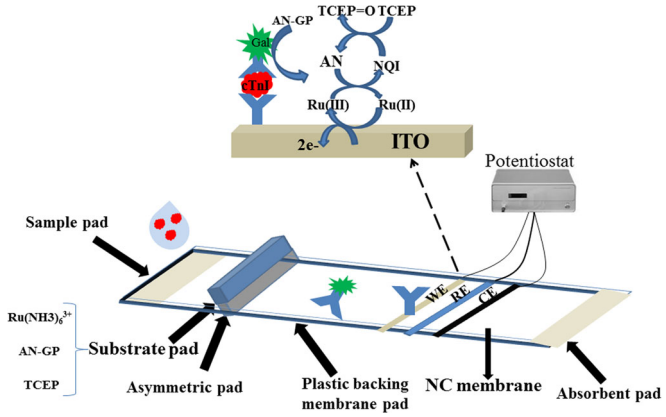

Figure 5. Schematic illustration of electrochemical-based LFIA for detection of TnI using ECC redox cycling.

This event was followed by a redox cycling and enzymatic reaction after unbound Gal-conjugated detection Abs were rinsed to remove their enzymatic reactions. AN-GP was converted to AN by Gal which triggers the fast ECC redox cycling of AN. When TCEP was oxidized to TCEP = O, naphthoquinone imine (NQI) was reduced to AN, followed by oxidization of AN to NQI in cyclic and consequently $Ru(NH3)_6^{3+}$ was reduced to $Ru(NH3)_6^{2+}$. On the surface of the ITO electrode, $Ru(NH3)_6^{2+}$ was oxidized to $Ru(NH3)_6^{3+}$, generating 2 free electron species. The three electrode system contained an ITO electrode as working electrode, an Ag/AgCl reference electrode, and an Ag counter electrode. It was observed that the selective ECC redox cycling which happened at the low applied potential (0.05 V vs. Ag/AgCl) minimized the interference of electroactive species such as acetaminophen, L-ascorbic acid (AA), and uric acid in serum. An LOD of 0.1 pg/mL for cTnI was reported within 11 minutes.[52] A simple LFIA that includes an asymmetric membrane pad and an ITO working electrode was proposed for efficient detection. The electrochemical detection in this particular LFIA was based on a selective ECC redox cycling including a novel incorporation of enzyme label (Gal), substrate (AN-GP), and product (AN) which resulted in rapid enzymatic reaction. This method effectively reduced the insignificant interference of electroactive species and background level in serum samples.

5. Conclusion

Fast detection of biomarkers for certain life-threatening health issues, specifically cardiovascular diseases, is crucial. Elevated amount of cTnI in the serum of the patient with or without AMI symptoms, is an important indicator of cardiac injuries. Rapid detection of cTnI saves the lives of patients with cardiac injuries. As reviewed in the current paper, LFIAs showed promising results in selectively, rapidly, and effectively detection of cTnI while they are inexpensive to produce. Recently, between different diagnostic tools, LFIAs have gained considerable attention due to fast and inexpensive diagnosis and prognosis ability, easy-to-use, accurate, reliable and lifesaving which is crucial for disease prevention and timely treatment. The LFIA strips can be easily designed and developed to further fulfill the needs in the clinics. They can be designed to detect a wide variety of analytes from microorganisms to peptides and proteins to detect or screen numerous types of health conditions. Developed LFIAs for cTnI detection enabled highly sensitive detection of cTnI in nanograms and picograms of cTnI in serum sample. By implementing nanoparticles as labels for generating and amplifying detection signals, highly sensitive LFIAs were developed with easy-to-read signals. Among all methods, colorimetric signal readouts have been highly explored and employed, since the test results are visible for the naked eye, and they eliminate the need for complicated and time-consuming interpretation of the result. More importantly, the results were ready in less than 10 minutes after the test was carried out, in most studies. These features make LFIAs ideal candidates for point-of-care detection of cTnI in patient samples.

Acknowledgments

The authors gratefully acknowledge the University of Medical Sciences, Mashhad, Iran for supporting this work. Y.N. Ertas acknowledges funding support from the 2232 International Fellowship for Outstanding Researchers Program of TÜBİTAK (Project No: 118C346).

Disclosure statement

The authors declare there is no Complete of Interest at this study.

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