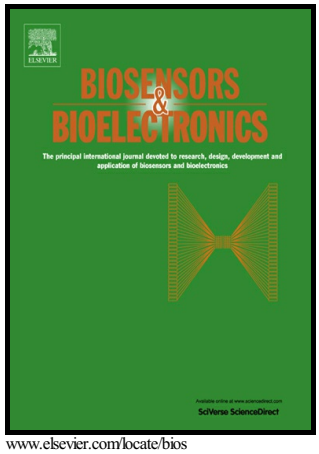

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Aptamer-based biosensors and nanosensors for the detection of Vascular Endothelial Growth Factor (VEGF): A review

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Abstract

Vascular endothelial growth factor (VEGF) is a key regulator of vascular formation and a predominant protein biomarker in cancer angiogenesis. Owing to its crucial roles in the cancer metastasis, VEGF detection and quantification is of great importance in clinical diagnostics. Today, there exist a wide variety of detection strategies for identifying many types of disease biomarkers, especially for VEGF. As artificial single-stranded DNA or RNA oligonucleotides with catalytic and receptor properties, aptamers have drawn lots of attention to be applied in biosensing platforms due to their target-induced conformational changes as well as high stability and target versatility. So far, various sensitivity-enhancement techniques in combination with a broad range of smart nanomaterials have integrated into the design of novel aptasensors to improve detection limit and sensitivity of analyte detection. This review article provides a brief classification and description of the research progresses of aptamer-based biosensors and nanobiosensors for the detection and quantitative determination of VEGF based on optical and electrochemical platforms.

Keywords: Vascular endothelial growth factor (VEGF), Aptamer, Aptasensor, Biosensor, Nanoparticle, Cancer biomarker

1. Introduction

Detection and quantification of disease biomarkers is of great importance for medical research, clinical diagnosis, and biomedical applications (Li et al. 2015a; Lin et al. 2015; Wu et al. 2016). For example, rapid detection of cancer-specific protein biomarkers in blood and serum samples significantly improves early diagnosis of cancers and the monitoring of subsequent therapeutic

treatments (Li et al. 2007; Lin et al. 2016). In this regards, protein biomarkers are becoming increasingly attractive targets for detection assays because these versatile biomolecules would be beneficial tools for understanding the pathophysiological processes of many diseases (Chattaraj et al. 2016; Pasquardini et al. 2015; Xu et al. 2017).

Vascular endothelial growth factor (VEGF), a hypoxia-inducible protein, is a key regulator of physiological vascular development during embryogenesis (vasculogenesis) as well as blood-vessel formation (angiogenesis) in adult tissues (Greenberg and Jin 2004; Olsson et al. 2006). In mammals, the VEGF family is comprised of five members, VEGFA, B, C, D and placenta growth factor (PLGF) (Ferrara et al. 2003; Goel and Mercurio 2013; Olsson et al. 2006; Simons et al. 2016). VEGFA as prototype member, which is often referred to as VEGF, has four different isoforms including $VEGF_{121}$, $VEGF_{165}$, $VEGF_{189}$, and $VEGF_{206}$ which are generated after alternative exon splicing of VEGFA gene (Hsu et al. 2016; Li et al. 2015b; Olsson et al. 2006). $VEGF_{165}$, as predominant isoform, accounts for a variety of human cancers development and metastasis (Li et al. 2017; Li et al. 2015b). VEGF mediates its activity through binding and stimulating two transmembrane endothelial receptor tyrosine kinases, VEGF receptors (VEGFR) types 1 and 2 (Ferrara et al. 2003). Moreover, as a signaling protein, it plays a pivotal role in pathological angiogenesis associated with many types of tumors and other conditions (Eskens and Verweij 2006; Goel and Mercurio 2013; Greenberg and Jin 2004; Olsson et al. 2006; Simons et al. 2016). In this regard, for the initiation of metastasis process, rapid growth of tumor cells needs independent blood supplies for more oxygen and nutrients resulting in the over-expression of VEGF. Thus, an elevated level of VEGF could be a possible indicator of cancers (Freeman et al. 2012; Hsu et al. 2016; Qureshi et al. 2015). In addition, down-regulation and increasing level of VEGF are observed in neurological disorders and inhibition of neuron degeneration, respectively (Fu et al. 2016). From a clinical point of view, VEGF has been used as a serum biomarker for various types of human diseases including cancers, proliferating retinopathy, rheumatoid arthritis, psoriasis and Parkinson's disease (Fu et al. 2016; Xu et al. 2017; Zhao et al. 2015; Zhu et al. 2017). Considering the above-mentioned critical roles of VEGF, this biomolecule has attracted remarkable attention for sensing and detecting through analytical sensing platforms as an important biomarker (Kwon et al. 2012; Lin et al. 2016; Shamsipur et al. 2015; Shan et al. 2017; Zhang et al. 2015).

Currently, there are several traditional detection and quantification methods for analyzing VEGF isoforms including enzyme-linked immunosorbent assays (ELISAs) as the gold standard of biomarker detection (Chattaraj et al. 2016; Tong et al. 2016), immunohistochemistry (Fu et al. 2016), fluorescent spectrometry (Lan et al. 2016) and radioimmunoassay (Zhao et al. 2011). However, these techniques often require sophisticated instrumentations with complicated protocols which make them expensive, labor-intensive, and time-consuming, and curb their use in real-time clinical diagnoses (Fu et al. 2016; Li et al. 2017; Qureshi et al. 2015). Thus, the development of rapid, highly sensitive and selective, cost-effective and label-free methods for the detection of VEGF in blood/serum is of great importance for different human disorders (Kwon et al. 2012; Kwon et al. 2010; Xu et al. 2017; Zhu et al. 2017). In recent years, scientists have employed a variety of new biosensing methods for the detection of VEGF. Nowadays, biosensors have achieved considerable success for VEGF detection (Turner 2013) due to sensitivity, specificity, cost-effectiveness, small target detection, and non-toxicity (Vigneshvar et al. 2016).

Biosensor technology has a wide range of applications with a central role in the medical diagnostics (Perumal and Hashim 2014; Vigneshvar et al. 2016). Generally, technical strategies involved in biosensors design can be broadly divided into label-free and label-based detection (Amouzadeh Tabrizi et al. 2015). In comparison with label-based systems, label-free biosensors are becoming a

consolidated approach for the detection of molecular interactions because of direct, real-time and improved multiplexed detection capabilities (Fan et al. 2008; Zanchetta et al. 2017). Incorporation of nanomaterials (NMs) and especially nanoparticles (NPs) with unique properties in biosensing design provides more simple, swift, sensitive and hybrid nano-biosensor platforms with synergetic properties and functions (Vigneshvar et al. 2016). Complexes of NPs with different bio-recognition elements (antibodies, peptide arrays, polymers, and aptamers) provide many versatile strategies for improving the performance of detection systems (Perumal and Hashim 2014).

Aptamers are single-stranded DNA (ssDNA), RNA or modified nucleic acids isolated by an in vitro selection process known as SELEX (systematic evolution of ligands by exponential enrichment) (Kimoto et al. 2016; Qureshi et al. 2015; Zhao et al. 2011; Zhu et al. 2017). Over the past decades, aptamers have been widely employed in developing various novel assay methods particularly biosensor platforms that are used for targeted drug delivery, analysis of biomolecules, and disease diagnosis (Li et al. 2017; Li et al. 2015a). Aptamers have recently been considered as highly promising biorecognition elements for recognizing protein biomarkers (Ko et al. 2013; Qureshi et al. 2015; Zhao et al. 2011) named as aptamer-protein complexes (Freeman et al. 2012; Fu et al. 2016). Aptamers exhibit unique features for bioanalytical and pharmaceutical applications including higher sensitivity and selectivity to their targets, smaller in size and complexity, easy and chemical synthesis, high stability even in non-physiological conditions, facile chemical modification, target versatility, resistance to degradation and denaturation, no batch-to-batch variation, and lower overall production costs (Cennamo et al. 2015; Pasquardini et al. 2015; Shan et al. 2017; Zhao et al. 2015; Zhao et al. 2011). In particular, aptamers undergo a conformational change when specifically interact with their targets that such structural change could omit additional labeling processes during monitoring of target binding events (Cho et al. 2012; Kopra et al. 2014; Li et al. 2015a; Yazdian-Robati et al. 2016). Given these attributes, aptamers are expected to become an alternative to antibodies (Chang et al. 2016; Kimoto et al. 2016; Taghavi et al. 2014).

Several aptamer-based sensors (aptasensors) have been designed for the detection of VEGF according to the availability of VEGF aptamers (Chen et al. 2014a). Some of the most important of these approaches include field-effect transistor (FET) (Kwon et al. 2012; Kwon et al. 2010; Lee et al. 2009), surface plasmon resonance (SPR) (Cennamo et al. 2015; Chen et al. 2014a; Chen et al. 2012; Li et al. 2007), fluorescence resonance energy transfer (FRET) (Freeman et al. 2012; Wang and Si 2013), surface-enhanced Raman scattering (SERS) (Ko et al. 2013; Zhao et al. 2015), and other optical and electrochemical methods (Chattaraj et al. 2016; Crulhas et al. 2017; Da et al. 2018; Kopra et al. 2014; Nonaka et al. 2012; Shan et al. 2017; Wong and Olivo 2014; Zhang et al. 2017). This wide range of VEGF aptasensors benefits from the target-binding affinity of aptamers and binding-induced conformational changes in aptamers to monitor VEGF-aptamer interactions using various electrical, optical and electrochemical signal-transduction approaches (Chang et al. 2016; Crulhas et al. 2017; Da et al. 2018; Freeman et al. 2012; Fu et al. 2016; Qureshi et al. 2015; Shamsipur et al. 2015; Zhang et al. 2015). This review mainly focuses on various developed aptamer-based platforms relying on two major technical strategies for the detection of VEGF. Optical-based VEGF aptasensors are covered first, followed by electrochemical sensing platforms. Moreover, we present the prominent roles of nanomaterials in the development of more sensitive and specific aptasensors emphasizing on the developed nanomaterial-based VEGF aptasensors (nano-aptasensors).

2. Optical VEGF aptasensors

Biosensors are classified by their method of signal transduction into electrochemical, optical, electronic, piezoelectric, gravimetric and pyroelectric biosensors (Damborský et al. 2016). Optical and electrochemical biosensors are two classes of widely used biosensing platforms (Vigneshvar et al.

2016). Optical biosensors are compact analytical devices briefly consist of a biorecognition sensing element producing a signal which is proportionate to the concentration of the desired analyte that recognized with an optical transducer system (Damborský et al. 2016). A broad range of biorecognition elements can be implicated in the fabrication of optical biosensors including enzymes, antibodies, antigens, receptors, nucleic acids, whole cells, and tissues in order to facilitate sensitive screening of a very wide range of most biologically relevant substances (Perumal and Hashim 2014). Recently, practical applications of optical biosensors are growing in many fields especially in healthcare, biomedicine, and biopharmaceutics (Damborský et al. 2016; Fan et al. 2008). Optical biosensors have many potential applications in portable biosensor systems and personalized medicine (Damborský et al. 2016). High specificity and sensitivity, cost effectiveness and small size of optical biosensors resulted in research and technological development in recent years because of their great potential for the direct, real-time and label-free detection of a wide variety of chemical and biological substances (Dey and Goswami 2011; Vigneshvar et al. 2016). In this section, the outstanding examples of various optical biosensors and nono-biosensors for VEGF detection were summarized based on employed transducing methods. Table 1 shows a list of literature reported optical VEGF aptasensors.

2.1. Luminescence-based VEGF aptasensors

Luminescence is a phenomenon where a substance emits light not resulting from heat (Murthy and Virk 2014). There are different types of luminescence methods classified according to the source of energy (trigger for the luminescence) in a wide variety of light emitting processes. For example, in chemiluminescence (CL), the source of energy for emission is chemical reactions. Furthermore, electrochemiluminescence (ECL) is produced during electrochemical reactions in solutions. Owing to considerable advantages such as high sensitivity, good selectivity and large linear quantitative range, luminescence-based methods, in their varied forms, have become increasingly popular for the design of sensitive and specific analytical techniques (Murthy and Virk 2014; Ronda and Srivastava 2006). In this context, several luminescence-based aptasensors for VEGF have been successfully developed.

In Kopra's work (2014), for the first time, a direct and non-competitive quenching resonance energy transfer (QRET) method using luminescent lanthanide-chelate labeled DNA aptamers was developed for VEGF quantification. QRET is an easy to use, rapid, and cost-effective single-label technique in which the detection relies on time-resolved luminescence (TRL) resulting from the interaction between a binding ligand labeled with lanthanide (Ln)(III) chelate, and a target molecule. Binding of the target molecule to Ln(III) chelate interferes with the quencher/ Ln(III) chelate interaction leading to the increased distance between the quencher and Ln(III) chelate, thus enabling luminescence signal formation (Kopra and Harma 2015). The QRET technique is more straight-forward than the fluorescence protection due to no requirement for the conformational changes of an aptamer and there are fewer manipulation steps for label conjugation (Harma et al. 2009; Kopra and Harma 2015). In this platform, an anti-VEGF aptamer conjugated to the Eu(III)-chelate was employed as a binding ligand. According to the process of QRET technique, the interaction of labeled anti-VEGF aptamer with VEGF resulted in protecting the Eu(III)-chelate from quenching by a soluble quencher molecule (Q5). Final luminescence signal increased with increasing VEGF concentrations. The limit-of-detection (LOD) of this single-label QRET method for VEGF was at a sub-nanomolar level with a dynamic range of 0.25–10 nM.

Recently, Lin et al. (2016) developed an aptasensor for label-free quantitative analysis of $VEGF_{165}$ using iridium (III) complex as a G-quadruplex-selective luminescent probe. Moreover, they constructed a hairpin-structured functional nucleic acid (FNA) consisting of a $VEGF_{165}$ aptamer, G-quadruplex sequence, and its complementary sequence. In the presence of $VEGF_{165}$, upon the

interaction of aptamer fragment of the hairpin with VEGF, the hairpin DNA was opened and the G-quadruplex structure was formed. Then the iridium (III) complex bound to G-quadruplex structure and consequently generated a luminescent signal. The hairpin structure cannot bind to iridium (III) complex when VEGF is absent (Fig 1A). This luminescence-based aptasensor can specifically detect $VEGF_{165}$ with low background, with a linear range (LR) of 0.52 nM to 78.0 nM and LOD of 0.17 pM.

To date, various powerful chemiluminescence-based analytical techniques have been developed, most of which benefit from the ease of setup, cost-effectiveness, high sensitivity and wide linear range of chemiluminescence assay (Li et al. 2015b; Pasquardini et al. 2015). In the context of VEGF, Pasquardini et al. constructed a high sensitive and low noise chemiluminescence aptasensor based on Single Photon Avalanche Diode (SPAD) detector for the detection of VEGF (Pasquardini et al. 2015). After VEGF capture by aptamer, which was coated on a silicon oxide surface, a specific primary antibody was attached to VEGF and then horseradish peroxidase (HRP)-conjugated secondary antibody recognized the primary antibody. Finally, captured VEGF was detected via chemiluminescence with a SPAD detector. The LOD was 58 pg mL^{-1} in Tris buffer and 1,715 pg mL^{-1} in human plasma spiked with VEGF. Li et al. (2015b) developed a label-free chemiluminescence VEGF aptasensor based on the aptamer-controlled noncovalent self-assembled porphyrin probe with a LOD 50 pM and a LR from 0 to 25 nM. They prepared a positively charged manganese porphyrin probe (Mn-PyP), which in its monomeric form, catalyzes luminol CL reaction. Because of its negative charge, anti-VEGF aptamer induced aggregation of Mn-PyP and, subsequently, suppression of Mn-PyP catalyzed CL reaction. Then, the addition of VEGF led to the release of the monomeric Mn-PyP and a turn-on CL signal is thus detected. This aptamer-based method did not require laborious and time-consuming covalent labeling process, providing a simple and sensitive label-free method.

Recently, a sensitive chemiluminescence-based dual-aptamer sandwich assay has been developed to target two different domains of $VEGF_{165}$ in a sandwich structure (Shan et al. 2017). In this assay, one biotinylated VEGF-binding aptamer was immobilized on streptavidin-coated magnetic beads (MBs) as capture aptamer and another aptamer was labeled with biotin for further alkaline phosphatase (ALP) conjunction that was attached to streptavidin (ALP-SA). Upon addition of $VEGF_{165}$ and formation of Apt-VEGF-Apt sandwich on MBs surface, ALP-SA catalyzed chemiluminescence reaction (Fig 1B). Their chemiluminescence aptasensor detected VEGF in standard spiked samples with a LOD of 1 ng mL^{-1} and linear range of 1 to 20 ng mL^{-1}. This assay has shown to have considerable performance for $VEGF_{165}$ detection in cell culture medium and may be further used for biosensing of cancer biomarkers.

A group reported an ECL method, for the first time, based on DNA aptamer–target recognition and T7 exonuclease (Exo) assisted cycling signal amplification for the detection of $VEGF_{165}$ (Zhang et al. 2015). They used CdS: Eu nanocrystals (NCs) as ECL substrate and modified glassy carbon electrode (GCE) with NCs for generation of ECL signal. Then, a VEGF-binding aptamer, a guanine (G)-rich ssDNA with aptamer recognition domain (S1), and a third single DNA sequence (S2) with recognition domains for VEGF aptamer and S1 were co-immobilized and formed as S1/S2/aptamer DNA strands on the surface of the CdS:Eu nanocrystals modified GCE. In the presence of VEGF, the aptamer was released from the electrode surface and formed aptamer–$VEGF_{165}$ complexes. Upon release of the aptamer, cyclic cleavage of the target DNA with T7 Exo was induced. On the other hand, G-rich ssDNA released on the CdS:Eu film and folded into G-quadruplex/hemin DNAzyme. Next, ECL of CdS:Eu nanocrystals were efficiently quenched through the formation of G-quadruplex/hemin DNAzyme (Fig 1C). On the basis of this approach, highly sensitive detection of VEGF occurred over the range of 1 pM to 20 nM and a detection limit of 0.2 pM.

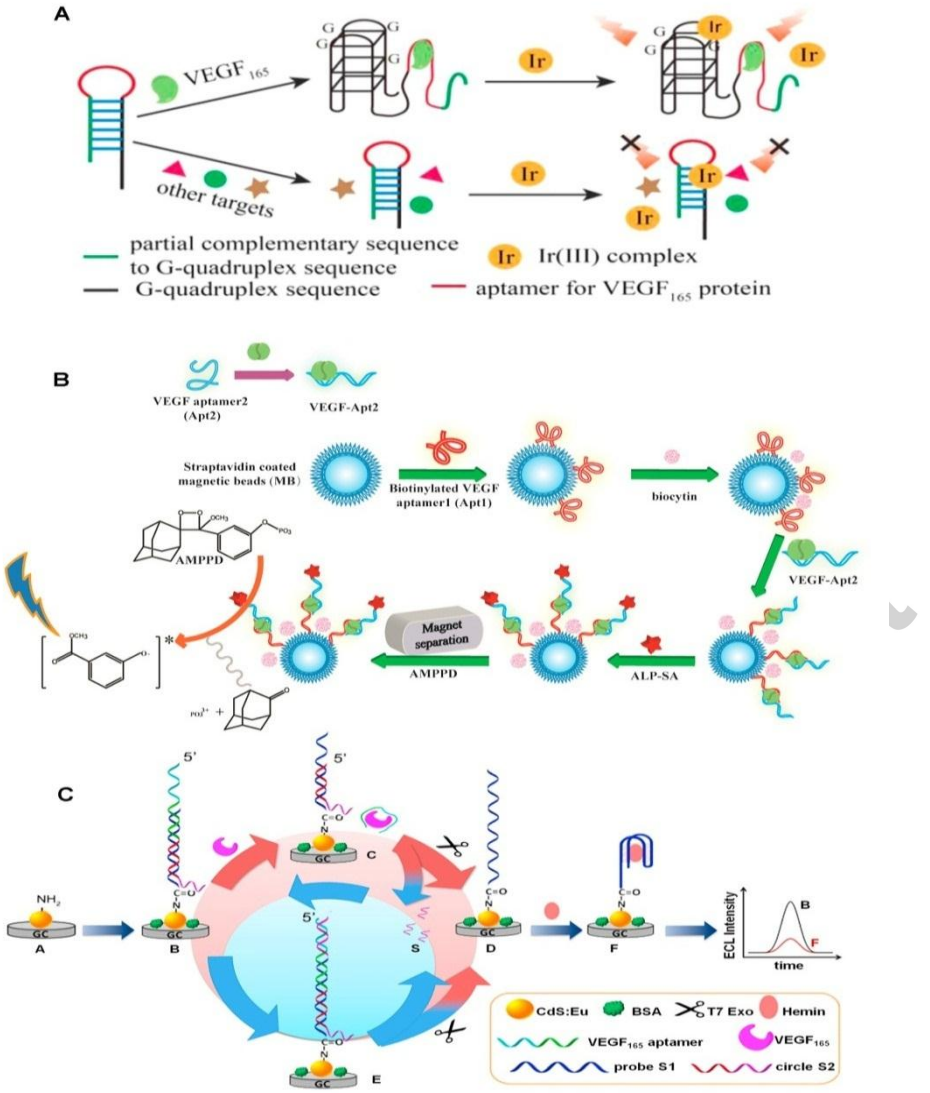

Fig 1. (A) A luminescence-based aptasensor based on G-quadruplex-selective luminescent probe developed for VEGF determination, (B) Schematic representation of a sensitive and selective chemiluminescence VEGF aptasensor, (C) Schematic illustration of an electrochemiluminescence for the detection of VEGF. Reprinted with permission from (Lin et al. 2016), (Shan et al. 2017) and (Zhang et al. 2015).

2.2. Fluorescence-based VEGF aptasensors

Generally, fluorescence is a form of luminescence (Valeur and Berberan-Santos 2011). Owing to its high sensitivity and efficiency, reproducibility, non-destructiveness, and rapid detection process, fluorescence-based detection is one of the most common optical techniques widely applied to develop various aptasensors (Fan et al. 2008; Feng et al. 2014; Lin et al. 2015; Wang et al. 2014). The first report of a highly sensitive and selective fluorescence VEGF aptasensor was reported by Wang et al. (2014), which was based on fluorescence polarization. In fluorescence polarization (FP) method, when fluorescent molecules were excited by polarized light, polarized emission would occur from the excited molecules. FP approaches can be applied for the rapid and quantitative analysis of diverse molecular interactions. In this study, a reagentless, simple and easy to use method was developed in an aqueous solution which only needed a single fluorescent dye attached to an anti-VEGF aptamer without any separation and washing steps. Upon the VEGF-aptamer binding, aptamer conformational change resulted in a G-quadruplex aptamer–VEGF complex which induced a significant increase in the FP signal. The detection limit of this aptasensor was 0.32 nM in aqueous solution.

In another study by Lin et al. (2015), a fluorescence-based aptamer system was developed for the determination of multiple proteins using microchip electrophoresis (MCE). The method relied on specific DNA aptamers against $VEGF_{165}$, PDGF-BB, and thrombin for detecting these proteins. In their system, aptamers were used as electrophoresis mobility modulators for electrophoretic separation and also as fluorescent affinity probe for fluorescence detection. SYBR gold was used as a fluorescent dye because it specifically binds to DNA or RNA and not to a protein, resulting in an enhanced selectivity of protein assay. Finally, they detected VEGF with a dynamic range of 5.00–150.0 nM and detection limit of 2.48 nM. It is suggested that this strategy can be used for analysis of other biological molecules having an aptamer binding capability.

Chattaraj et al. (2016) designed a fluorescence-based detection strategy for in-solution biosensing. They hypothesized that lipid-stabilized oil droplets containing fluorogenic redox molecules would be aggregated when analyte came into close proximity. Based on this hypothesis, they reduced the hydrophobic cyanine dye 1,1′-dioctadecyl-3,3,3′3′ tetramethylindocarbocyanine perchlorate (DiI) with sodium borohydride to form a non-fluorescent analog (HDiI) which subsequently can be oxidized with p-fluoranil, a hydrophobic quinone derivative, to fluorescent DiI. Then, they loaded HDiI and p-fluoranil into NEOBEE oil nanodroplets that were stabilized with a monolayer of 1, 2-dipalmitoyl-sn-glycero-3-phosphocholine (DPPC), 1, 2-distearoyl-sn-glycero-3-phosphoethanolamine (DSPE)-polyethylene glycol (PEG), and DSPE-PEG-anti-VEGF aptamer. Addition of VEGF caused aggregation of droplets, oxidizing the HDiI with p-fluoranil and production of fluorescent DiI leading to the emission of red fluorescence that was recorded with Nanoparticle Tracking Analysis (NTA). The aptasensor exhibited a detection limit of 100 fM with a dynamic range between 100 fM and 10 pM.

Recently, a new fluorescence-based VEGF aptasensor has been developed based on switching of DNA assembly structure and isothermal amplification (Li et al. 2017). This system took advantage of an enzymatic isothermal amplification named EXPAR, an exponential amplification reaction, to construct an aptamer-based VEGF sensing method. EXPAR is an emerging isothermal nucleic acid amplification method that combines polymerase strand extension with single-strand nicking which efficiently amplifies short oligonucleotides within minutes. Owing to its advantages such as isothermal nature, high amplification efficiency, and rapid amplification kinetics, this technique has a high potential for molecular diagnostics (Mok et al. 2016). Briefly, this biosensing platform consists of an aptamer DNA, a primer DNA, a protector DNA and a template DNA that form DNA assembly. Following the addition of VEGF, it induces an aptamer structure switching (structural modification of the DNA assembly) that makes running enzymatic isothermal amplification reaction to produce ssDNA. Finally, a toehold mediated DNA strand displacement reaction occurs using 5 nt unhybridized region of the fluorescent/quencher labeled probe leading to an increase in fluorescent signal. They reported a linearity range of 5–400 pg mL^{-1} and a detection limit of 3.5 pg mL^{-1} for VEGF detection.

Mita et al. (2014) investigated a VEGF Fluorescence aptasensor based on peptide nucleic acid (PNA) and bound/free separation system. They designed a capture PNA (CaPNA), with a complementary sequence to VEGF aptamer, and immobilized it on NeutrAvidin beads. Moreover, VEGF aptamer was labeled with fluorescein. Briefly, in the presence of VEGF, upon formation of aptamer–VEGF complex, subsequent stabilization of fluorescein-labeled VEGF aptamer and steric hindrance of VEGF inhibited aptamer-CaPNA hybridization and finally resulted in increased fluorescence intensity. On the contrary, the absence of VEGF decreased fluorescence intensity due to the CaPNA hybridization with the fluorescein-labeled VEGF aptamer. By means of this strategy, the LOD of for VEGF was determined as 25 nM in a linear range of 5 nM to 50 nM.

In another study, Sosic et al. (2013) designed a VEGF fluorescence aptasensor based on a sandwich aptamer microarray (SAM). They utilized two fluorescently labeled DNA aptamers, Vap7 and VEa5, simultaneously recognized different epitopes of VEGF and the formation of a ternary complex was analyzed by electrophoretic mobility shift assay (EMSA). Moreover, authors evaluated the performance a multiplex system involving prepared VEGF aptasensor with previously reported thrombin aptasensor for the simultaneous detection of $VEGF_{165}$ and thrombin.

Despite studies demonstrating that both luminescence and fluorescence biosensors are useful for VEGF detection, the intrinsic fluorescence of some proteins in serum may interfere with the outcome of the sensor and thus the fluorophores with time-resolved fluorescence properties should be used (Alexiev et al. 2017).

2.3. Colorimetric-based VEGF aptasensors

Colorimetric-based detection strategies have been widely applied for developing simple and sensitive aptasensors (Du and Dong 2017; Feng et al. 2014; Zhang et al. 2017). In a colorimetric process, a color change is instantly observed in the presence of target analytes with the naked eye without the need for any measuring device (Liu et al. 2009; Seo and Gu 2017; Yoo and Lee 2016).

Nowadays, immuno-nucleic acid amplification strategies have attracted great attention. Hybridization chain reaction (HCR) is an enzyme-free amplification process with a simple protocol and excellent amplification efficiency performed under isothermal conditions (Choi et al. 2014; Evanko 2004). Owing to these advantages, HCR has drawn increasing attention for application in biosensing platforms (Bi et al. 2017). In this aspect, two similar studies reported colorimetric aptasensors for the detection of VEGF based on immuno-HCR (iHCR) (Xu et al. 2017; Zhu et al. 2017). The aptasensor planned by Xu et al. (2017) consisted of VEGF aptamer, VEGF antibody and glucose oxidase (GOx)-functionalized ssDNAs. The antibody was immobilized on the microplate and could capture VEGF. Then, aptamer bound VEGF formed the sandwich structure. Next, hybridization chain reaction was initiated using GOx-functionalized ssDNAs and the aptamer as a primer. Finally, aggregation of GOx on the microplate led to the catalysis of glucose oxidation which promoted the pH change. In this study, generated detection signal was collected by a handheld pH meter. Moreover, after addition of pH indicator to the reaction solution, the color change was distinguishable by naked-eyes (Fig 2A). The detection limit of this aptasensor for VEGF was 0.5 pg mL^{-1} measured by handheld pH meter and 1.6 pg mL^{-1} of VEGF could be read by the naked eyes. Applying inexpensive and portable pH meter for fabrication of this aptasensor, has made it a useful device for the point-of-care (POC) diagnosis.

Based on a similar principle, Zhu et al. (2017) developed a portable and sensitive colorimetric aptamer-based immunosensor for the detection of VEGF. The method relied on HCR and portable glucose meter (PGM) (Fig 2B). Similar to previous work, immobilized antibody on the microplate captured the VEGF and formed aptamer/VEGF/antibody complex after addition of VEGF aptamer. Moreover, they used two invertase-functionalized auxiliary probes. One of them had a complementary sequence with an 18-nt elongated strand (as the primer) at the 3′ end of the aptamer leading to the generation of invertase-concatamers. Next, upon adding sucrose, invertase on the concatamers catalyzed the conversion of sucrose into glucose. Finally, the generated glucose was detected by a portable personal glucometer. They reported a detection limit of 1.2 pg mL^{-1} for VEGF by means of this immuno-aptasensor.

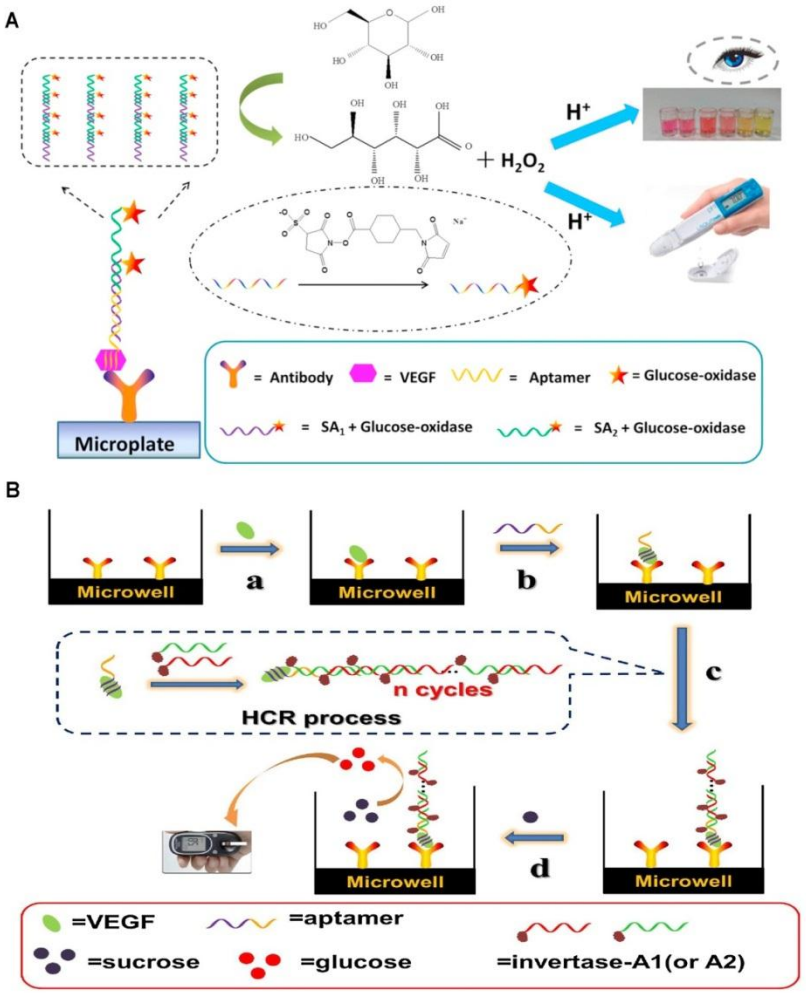

Fig 2. Examples of portable and sensitive colorimetric aptasensors developed for VEGF detection. Reprinted with permission from published paper (Xu et al. 2017) and (Zhu et al. 2017).

Zhang et al. (2017) used strand displacement amplification (SDA), an isothermal nucleic acid amplification technique, to develop a label-free colorimetric aptasensor for the sensitive detection of VEGF. In their study, aptamers were designed as hairpin probes to bind to VEGF. Briefly, the function of developed aptamer was based on initiation of SDA reaction upon specific binding of the hairpin aptamer to VEGF, generation of G-quadruplex DNAzyme and subsequent conversion of colorless $ABTS^{2-}$ to a green $ABTS^{\bullet-}$ in the presence of H_2O_2 and hemin. VEGF was detected with a LOD 1.70 pM and a dynamic range from 24.00 pM to 11.25 nM.

In comparison with other sensing assays, reported VEGF colorimetric aptasensors can be applied in the development of cost-effective detection method because of inclusion of an isothermal and portable manner in its design.

2.4. SPR-based VEGF aptasensors

SPR is one of the powerful label-free techniques for the measurement of ligand-analyte interactions. SPR-based sensors have gradually become the predominant optical aptasensors (Damborský et al. 2016; Fan et al. 2008). Basically, SPR phenomenon senses any changes in refractive index proportional to the mass of chemical and biological species which were immobilized on the sensor

surface (Feng et al. 2014; Špačková et al. 2016). Currently, SPR biosensors have made great advances in numerous fields, especially in medical diagnostics (Du and Dong 2017). SPR imaging (SPRI) sensor technology is established by merging of SPR and imaging techniques which can be used for simultaneous detection of multiple interactions (Chen et al. 2012; Damborský et al. 2016; Li et al. 2007; Wong and Olivo 2014). Based on this idea, an enzymatically amplified SPRI methodology was established to detect protein biomarkers by a combination of RNA aptamers and antibodies (Li et al. 2007). In this strategy, after formation of a surface aptamer-protein-antibody complex, an antibody-conjugated HRP enzyme amplified the response signal. In the demonstration of their experiment, VEGF was detected with an LOD of 1 pM. Sensitivity of this method was comparable with the sensitivity observed in fluorescence antibody sandwich assays. In another study, chen et al. (2012) reported a similar SPRI method for multiplexed protein biosensing. They used gold thin films in a microfluidic format for one-step, on-chip synthesis of RNA aptamer microarrays from DNA microarrays by the surface transcription reaction of T7 RNA polymerase. Then, they monitored bioaffinity adsorption of VEGF onto RNA aptamer microarrays (Fig 3). Another group examined a rolling circle amplification (RCA)-assisted SPR strategy for the detection of VEGF. Briefly, RCA is an isothermal enzymatic process for replication of long ssDNAs. In this cost-effective and real-time method, two DNA aptamers targeted different domains of VEGF. The RCA process amplified the SPR signal and increased sensitivity and specificity of VEGF detection with an LOD of 100 pg mL^{-1} (Chen et al. 2014a).

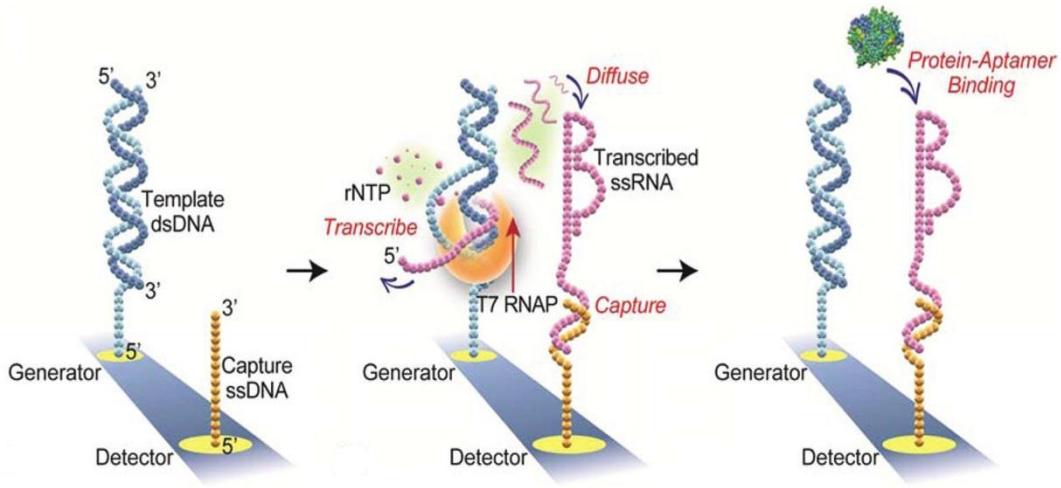

Fig 3. Schematic of SPR-based VEGF aptasensors for the detection of VEGF. Reprinted with permission from (Chen et al. 2012), copyright (2017) American Chemical Society.

The sensitivity of the SPR technique for the detection of VEGF by aptamers has been demonstrated by Cennamo et al. (2015). They functionalized a simple and low-cost biosensor with aptamer layer as biorecognition elements and plastic optical fiber (POF) with a planar gold layer as a light guiding region. Moreover, their aptasensor showed increased sensitivity and stability after applying mercaptoethanol as a passivating agent. VEGF detection limit of this SPR-based aptasensor based on aptamer alone and aptamer-mercaptoethanol was 3 nM and 0.8 nM, respectively.

In this section, several optical VEGF aptasensors have been described by different performance (Table 1). In the past decade, various research groups attempted to improve the performances of these aptasensors by applying nanomaterials.

Table 1: Comparison of analytical performance for reported optical VEGF aptasensors.

Detection Method	Strategy	Recognition motif	Limit of detection (LOD)	Linear range (LR)	Type of VEGF	Ref.
Luminescence	Quenching resonance energy transfer (QRET) assay	DNA aptamer	–	0.25–10 nM	–	(Kopra et al. 2014)
	G-quadruplex-selective luminescent iridium (III) complex	DNA aptamer	0.17 pM	0.52 nM to 78.0 nM	$VEGF_{165}$	(Lin et al. 2016)
	Single photon avalanche diode (SPAD)	DNA aptamer	58 pg mL^{-1}	–	$VEGF_{165}$	(Pasquardini et al. 2015)
	Aptamer controlled catalysis of a Porphyrin Probe	DNA aptamer	50 pM	0 to 25 nM	$VEGF_{165}$	(Li et al. 2015b)
	Dual-aptamer-based sandwich assay	DNA aptamer	1 ng mL^{-1}	1 to 20 pg·mL^{-1}	$VEGF_{165}$	(Shan et al. 2017)
	T7 exonuclease-assisted cycling signal amplification /G-quadruplex/hemin DNAzyme	DNA aptamer	0.2 pM	1 pM to 20 nM	$VEGF_{165}$	(Zhang et al. 2015)
Fluorescence	Fluorescence polarization based on recognition reaction	DNA aptamer	0.32 nM	0.32 to 5.0 nM	$VEGF_{165}$	(Wang et al. 2014)
	Microchip electrophoresis	DNA aptamer	2.48 nM	5.00–150.0 nM	$VEGF_{165}$	(Lin et al. 2015)
	Fluorogenic hydrocyanine/quinone reporter pairs/nanodroplet association	DNA aptamer	100 fM	100 fM and 10 pM	$VEGF_{165}$	(Chattaraj et al. 2016)
	DNA assembly structure switching and isothermal amplification	DNA aptamer	3.5 pg mL^{-1}	5 pg mL^{-1} to 400 pg mL^{-1}	$VEGF_{165}$	(Li et al. 2017)
	PNA-Based bound/free separation system	DNA aptamer	25 nM	5 nM to 50 nM	–	(Mita et al. 2014)
Colorimetric	Sandwich aptamer microarray	DNA aptamer	–	–	$VEGF_{165}$	(Sosic et al. 2013)
	Immuno-HCR	DNA aptamer	0.5 pg mL^{-1}	0.8–480 pg mL^{-1}	–	(Xu et al. 2017)
	Immuno-HCR	DNA aptamer	1.2 pg mL^{-1}	3–100 pg mL^{-1}	$VEGF_{165}$	(Zhu et al. 2017)
	Strand displacement amplification (SDA)	DNA aptamer	1.70 pM	24.00 pM to 11.25 nM	$VEGF_{165}$	(Zhang et al. 2017)
Surface plasmon Resonance/imaging (SPR/I)	RNA aptamer/protein biomarker/ HRP-conjugated antibodies sandwich structure	RNA aptamer	1 pM	–	$VEGF_{165}$	(Li et al. 2007)
	on-chip synthesis/ RNA aptamer microarray/ microfluidic format	RNA aptamer	–	–	$VEGF_{165}$	(Chen et al. 2012)
	Rolling circle amplification (RCA)	DNA aptamer	100 pg mL^{-1}	100 pgmL^{-1} to 1 µg mL^{-1}	$VEGF_{165}$	(Chen et al. 2014a)
	plastic optical fiber (POF)-SPR	DNA aptamer	3 nM	–	$VEGF_{165}$	(Cennamo et al. 2015)

3. Optical VEGF nano-aptasensors

Considering the unique features of nanomaterials, several novel electrochemical and optical biosensors were developed for the detection of different biomaterials (Amouzadeh Tabrizi et al. 2017; Chang et al. 2016; Freeman et al. 2012; Lee et al. 2009; Li et al. 2015a; Ravalli et al. 2015; Wu et al. 2016). A number of nanomaterials have been used for immobilization of biomolecules in biosensor development and one of their phenomenal roles is to provide considerable sensitivity and specificity for biosensor devices (Vigneshvar et al. 2016). On the other hand, incorporation of these nanocomponents in bio-analytical devices has potential advantages for lowering the limit of detection and enhancing the ability of single molecule detection (Mokhtarzadeh et al. 2015; Turner 2013). In this context, a combination of nanomaterials with fluorescence methods yielded in more sensitive and applicable optical biosensors (Dey and Goswami 2011). For instance, some nanomaterials such as gold nanoparticles and graphene oxide (GO) as super-quencher can reduce the background signals and therefore improve the sensitivity of fluorescence aptasensors compared to traditional organic quenchers (Li et al. 2015a).

Among various metal NPs, gold nanoparticles (AuNPs) have been broadly used in bioanalytical methods (Jia et al. 2016). Outstanding characteristics of AuNPs such as high stability, large surface area, considerable plasmonic properties, controllable morphology, biocompatibility, and their ability to be easily functionalized make these nanomaterials excellent transduction platforms for biosensing (Kumar et al. 2015; Omidfar et al. 2013). Moreover, because of optical, catalytic and conductivity properties of AuNPs, a wide variety of optical AuNPs-based aptasensors have been developed for the detection of VEGF (Lan et al. 2017; Viswambari Devi et al. 2015). It should be noted that when AuNPs-based sensors were used for the detection of targets in complex samples like serum, a problem usually appears called "corona shield effect". The use of PEG molecules in the structure of biosensors can address this issue (Dreaden et al. 2012). In this context, numerous optical nano-aptasensors have been proposed for VEGF detection (Table 2).

Table 2. Optical VEGF nano-aptasensors (nanomaterial-based optical VEGF aptasensors)

Detection Method	Strategy /Nanomaterial	Recognition motif	LOD	Linear range	Type of VEGF	Ref.
Luminescence	Upconversion nanoparticles (UCNPs) luminescence assay	DNA aptamer	6 pM	50 pM to 2000 pM	–	(Lan et al. 2016)
Fluorescence	Peroxidase-like activity/Apt–Au NPs/BiOCl nanocomposites	DNA aptamer	0.5 nM	-	$VEGF_{165}$	(Hsu et al. 2016)
	GO-based FRET nano-aptasensor	DNA aptamer	2.56×10^{-10} M	5×10^{-10} to 5×10^{-9} M	$VEGF_{165}$	(Wang and Si 2013)
	GO-based fluorescence nano-aptasensor using enzyme-assisted target-recycling signal amplification	DNA aptamer	1 pM	5-200 pM	$VEGF_{165}$	(Li et al. 2015a)
	FRET based QD-nano aptasensor, FRET/CRET/ Exonuclease III-based amplification	DNA aptamer	5 pM for Exo III amplification	–	$VEGF_{165}$	(Freeman et al. 2012)
Colorimetric	Colorimetric assay based on AuNPs / target-induced activation of aptazyme	DNA aptamer	0.1 nM	0.1 nM to 40 nM	$VEGF_{165}$	(Wu et al. 2016)
	Colorimetric assay based on AuNPs/target-assisted cascade amplification	DNA aptamer	185 pM	up to 7.4 nM	-	(Chang et al. 2016)
SERS/LSPR	SERS-based sandwich immunoassay using SEHGNs and a gold-patterned microarray	DNA aptamer	~1–10 pg mL^{-1}	0.1 to 1000 ng mL^{-1}	-	(Ko et al. 2013)
	SERS/AgNPs ornamented–AuNPs pyramids	DNA aptamer	22.6 aM	0.01–1.0 fM	$VEGF_{165}$	(Zhao et al. 2015)
	surface-enhanced Fluorescence/ LSPR using gold nanoplasmonic particles (GNPs)	RNA aptamer	-	25 pg mL^{-1} to 25 μg mL^{-1}	$VEGF_{165}$	(Cho et al. 2012)

3.1. Luminescence-based VEGF nano-aptasensors

Upconversion nanoparticles (UCNPs) are emerging as a new class of imaging agents that convert low excitation energy photons into visible emissions (Song et al. 2017). UCNPs have drawn much attention for bioimaging purposes in the medical field (Chen et al. 2014b; Wang et al. 2011). Recently, one group designed a luminescence aptasensor based on UCNPs and sequence fragment-linked of target-induced aptamer for the detection of VEGF (Lan et al. 2016). They synthesized $NaYF_4$:Yb^{3+}, Er^{3+} UCNPs by thermal decomposition of rare-earth stearates as precursors in a high-temperature system. Then, a VEGF aptamer was split into two fragments as capture probe and signal probe. Moreover, they used UCNPs as luminescence labels for modification of signal probe aptamer. First, The VEGF bound to the capture probe that was immobilized on the surface of a microplate. Upon addition of signal probe and binding to the VEGF-loaded capture probe, the formation of UCNPs-aptamer complex led to the detection of upconversion luminescence (UCL) signal. On the other hand, in the presence of VEGF, no UCNPs-aptamer complex was formed and no UCL signal

was detected. On the basis of this approach, VEGF was detected with an LOD of 6 pM and liner detection range from 50 pM to 2000 pM.

3.2. Fluorescence-based VEGF nano-aptasensors

In a recent study, Hsu et al. (2016) designed an Au-based aptasensor for the detection of $VEGFA_{165}$. They immobilized aptamer-modified gold nanoparticles (Apt-AuNPs) on bismuth oxychloride (BiOCl) nanosheets and constructed Apt-AuNPs/BiOCl nanocomposite. In this work, two aptamers were used in the Apt-AuNPs/BiOCl nanocomposite for high-affinity binding of aptamer-VEGF. In the absence of VEGF, the prepared aptasensor showed high peroxidase-mimicking activity and converted Amplex Red (AR) to fluorescent resorufin in the presence of H_2O_2. When WEGF was added to the assay system, the formation of aptamer-VEGF complexes led to the inhibition of catalytic activity of the nanocomposite followed by a decreased fluorescence response. This nano-aptasensor showed high sensitivity and selectivity for $VEGFA_{165}$ with an LOD of 0.5 nM.

Graphene as a carbon-based nanomaterial has attracted great attention owing to its unique electrical and optical properties, biocompatibility and considerable thermal and electrical conductivity (Pan et al. 2017). Graphene oxide, a single monomolecular layer of graphite and oxygen and as a water-soluble derivative, has found promising biosensing applications (Wang and Si 2013). As mentioned earlier, GO has super-quencher properties and can be incorporated into fluorescence aptasensors to increase sensitivity and reduce background signals (Charbgoo et al. 2016; Li et al. 2015a). In this line, two studies described GO-based fluorescence VEGF aptasensors.

Wang et al. (2013) developed a GO aptasensor relied on FRET for sensitive and specific detection of VEGF. FRET is a distance-dependent physical phenomenon by which photo-excitation energy is transferred nonradiatively between two fluorescent molecules (donor and acceptor) that are sufficiently close (Feng et al. 2014; Wang and Si 2013). FRET-based sensors as typically labeled fluorescence sensing platforms have been used extensively in biomedical research and drug discovery today (Chen et al. 2014b; Piston and Kremers 2007). Employing this approach, adsorption of fluorescent dye-labeled-VEGF aptamer on the surface of GO sheets led to the fluorescence quenching of the dye due to the highly effective FRET from the dye to GO. Following the formation of VEGF-aptamer complexes, aptamers released from the GO sheets and enabled detection of the restored fluorescence signal of the dye. The LOD of this cost-effective GO-based nanosensor for VEGF was determined to be 2.56×10^{10} M.

In another study, Li and co-workers (2015a) developed a GO-based fluorescence aptasensor using enzyme-assisted target-recycling signal amplification strategy for detection of VEGF and ATP (Fig 4A). In their design, they used single labeled molecular aptamer beacon cleavage site for the nicking endonuclease as the recognition element and mediating signal amplification and GO as quencher. In the absence of VEGF, the fluorescently-labeled VEGF aptamer was adsorbed on the GO nanosheet leading to its quenching. Conversely, in the presence of VEGF, aptamer-VEGF complexes were recognized by nicking enzyme and led to the cleavage of fluorescently-labeled VEGF aptamer into two short DNA fragments which had a weak affinity toward GO nanosheets. This phenomenon resulted in the weakness of the quenching of the fluorescent dye and consequently enhancement of the fluorescence intensity. Taking advantages of molecular aptamer beacon and nicking enzyme, can resolve the problem of false positive signals and by using quenching properties of GO the background signals can be reduced. The developed aptasensors exhibited a linear range of 5-200 pM and LOD of 1 pM toward VEGF. A drawback of biosensing methods employing GO is alteration of the sensitivity of the sensing platform by their interaction with serum proteins. Pretreatment of GO with albumin or PEG may be alternative strategies to overcome this limitation (Chung et al. 2013).

Quantum dots (QDs) are considered for biosensing strategy because they can be modified in terms of biocompatibility, sizes, charges, stability, surface coating and core/shell structure (Jin et al. 2011). Freeman et al. (2012) introduced a series of CdSe/ZnS semiconductor QDs-based optical aptasensors for VEGF detection based on the FRET and chemiluminescence resonance energy transfer (CRET). In the FRET-based aptasensors, they used modified CdSe/ZnS QDs with anti-VEGF aptamer and black-hole quencher (BHQ2)-functionalized nucleic acid and then measured increase in chemiluminescence of QD after addition of VEGF. In second strategy, they used a split anti-VEGF aptamer and linked these two aptamer subunits to QD and Cy5, a fluorescent dye, and then observed FRET phenomena from QD to the dye in the presence of VEGF subsequent to stabilization of aptamer subunits-VEGF complex. Since some aptamers could fold into the G-quadruplex structures after conjugation with their targets, they used hemin/G-quadruplexes as color or chemiluminescence labels because hemin/G-quadruplexes showed a catalytic activity similar to the activity of HRP enzyme. Moreover, QDs/hemin/G-quadruplex conjugates were fabricated for the detection of aptamer-substrate complexes via developed CRET-based aptasensor. Furthermore, they fabricated a QDs-based optical aptasensor with amplified fluorescence sensing using exonuclease III (Exo III)-catalyzed regeneration of VEGF with a detection limit of 5 pM and 50 pM in buffer solution and human serum samples, respectively. The toxicity, intrinsic blinking, chemical instability and low fluorescence are limiting factors using QDs in biosensing methods (Jin et al. 2011).

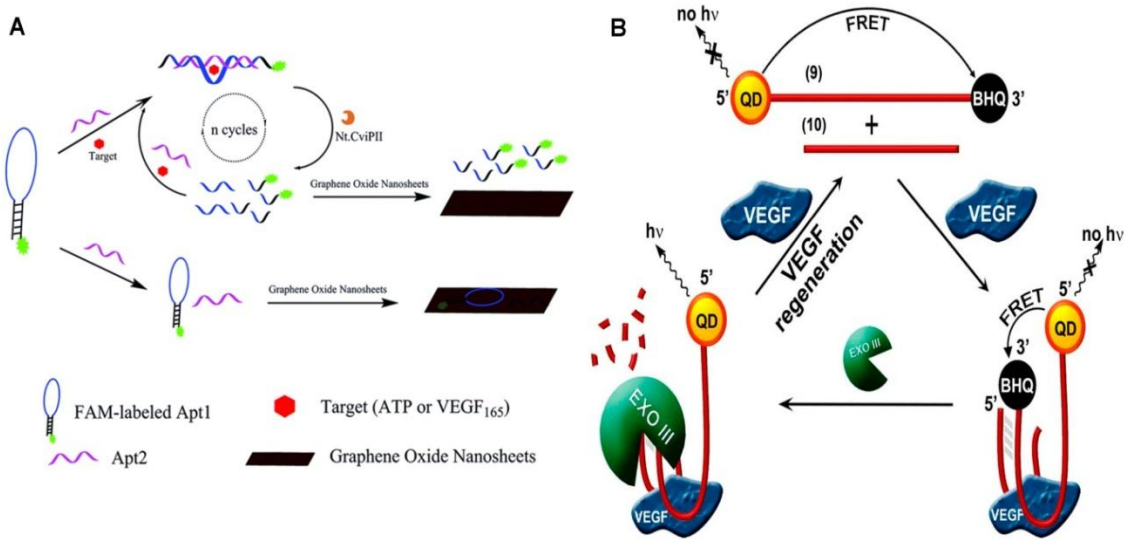

Fig 4. (A) GO-based fluorescence aptasensor using enzyme-assisted target-recycling signal amplification strategy for the detection of VEGF, (B) QDs-based optical sensing of $VEGF_{165}$ by the formation of a VEGF−aptamer subunits supramolecular structure that stimulates a FRET process. Reprinted with permission from (Li et al. 2015a) and (Freeman et al. 2012); Copyright (2017) American Chemical Society.

3.3. Colorimetric-based VEGF nano-aptasensors

Colorimetric aptasensors have mainly employed AuNPs and G-quadruplex/hemin HRP-mimicking DNAzyme as signal reporters in their design (Feng et al. 2014; Ramezani et al. 2015). Owing to the fact that obvious color change can be observed in dispersed and aggregated conditions, numerous AuNPs-based colorimetric methods have conducted for the detection of VEGF proteins (Du and Dong 2017; Liu et al. 2009). In this regard, a hairpin-structure aptazyme was developed consisting of a VEGF aptamer, DNAzyme, and a short stem sequence (Wu et al. 2016). In this system, a linker attached to the AuNPs was designed for cross-linking of gold nanoplasmonic particles (GNPs) as well as acted as a substrate sequence of the DNAzyme. In the presence of VEGF, aptamer-VEGF

interaction led to the conformational change of aptazyme activating the DNAzyme. Next, DNAzyme cleaved the AuNPs-attached linkers and thus no cross-linked AuNPs was observed and the color of GNPs solution remained red. In contrast, the color of AuNPs changed from red to blue in the absence of VEGF and subsequent cross-linking of AuNPs. Simple operation is an advantage of this aptasensor which is because of coincident accomplishment of target recognition and signal amplification, visual detection of the target using AuNPs, and thermo-stability. Based on this enzyme-free amplified colorimetric assay, VEGF was detected in a linear range from 0.1 nM to 40 nM and LOD of 0.1 nM.

Lately, Cheng et al. used a bi-functional hairpin probe consisting of VEGF aptamer sequence and an initiation sequence which was designed to trigger the nonlinear HCR (NLHCR) as the branched DNA amplification strategy (Chang et al. 2016). Binding of VEGF to its corresponding aptamer results in a cascade amplification reaction of NLHCR which in turn forms a DNA dendritic nanostructure consisting of two double-stranded substrate DNAs and two auxiliary ssDNAs as assembly components. This phenomenon brought about the adsorption of the DNA dendrimer on the DNA sensing probe-stabilized AuNPs leading to the aggregation and color change of AuNPs from red to reddish-violet. Conversely, in the absence of VEGF, AuNPs became stable without color change. The results showed that under optimum conditions, the LOD was 185 pM.

3.4. SERS/LSPR-based VEGF nano-aptasensors

Surface-enhanced Raman spectroscopy or surface-enhanced Raman scattering (SERS) is a powerful analytical technique for the sensitive and selective sensing of chemical and biological molecules (Stiles et al. 2008; Zhao et al. 2015). Based on the principle of SERS, Raman scattering intensity of molecules could be enhanced by adsorption onto the metal surfaces. Gold and silver nanoparticles, as SERS-active surfaces, have been widely used in SERS-based detection methods (Feng et al. 2014; Laing et al. 2017). One group developed an immuno-aptasensor for the detection of VEGF based on SERS technique, silica-encapsulated hollow gold nanospheres (SEHGNs) and a gold-patterned microarray (Ko et al. 2013). In this designed SERS-based sandwich immunoassay, VEGF antibodies were immobilized on a gold-patterned microarray which could capture VEGF. Then, VEGF DNA aptamer-conjugated SEHGNs were added which formed sandwich immune-complexes. In this aptasensor, the aptamer-conjugated SEHGNs were used as SERS-encoding nanoprobes while the gold-patterned microarray was used as a SERS substrate. Finally, SERS peak intensity of the selected Raman reporter molecules adsorbed onto the surfaces of HGNs was used for the quantitative analysis of VEGF. This aptasensor exhibited a detection limit of 1–10 pg mL^{-1}.

In another study, a SERS-based aptasensor using AuNPs was reported by Zhao et al. (2015). To implement this system, they employed VEGF aptamer and its partial complementary sequence to assemble silver nanoparticle (AgNPs) ornamented-AuNPs pyramids (Ag–Au Pys) as a SERS substrate. Moreover, 4-nitrothiophenol (4-NTP) as a Raman reporter was used to modify AgNPs. In the absence of VEGF, aptamer-modified AgNPs were combined with Au pyramid structure led to a strong SERS signal. Upon the addition of VEGF and specific aptamer-VEGF binding, AgNPs were released from the scaffold of the Au pyramid led to the weakness of SERS intensity. Finally, the intensity of SERS signal was used to quantify the concentration of VEGF (Fig 5A). The developed aptasensor detected VEGF in the range of 0.01–1.0 fM with LOD of 22.6 aM.

Cho and co-workers (2012) reported a single-step aptamer-based surface-enhanced fluorescent (SEF) method based on aptamer-target recognition and nanoplasmonic fluorophore interaction for the detection of VEGF. In the developed aptasensor, negatively charged, citrate-stabilized GNPs were fixed on a positively charged aminosilane-coated glass slide. Also, GNPs covered with positively charged poly-L-lysine (PLL) to electrostatically attract and stabilize VEGF-specific aptamer. Moreover, aptamers were conjugated with a fluorescent molecule, Cy3B (Cyanine Dye 3B), to

directly monitor the aptamer-VEGF binding without further labeling step. In the absence of VEGF and subsequent electrostatic binding of aptamer with PLL on the surface of GNPs, metal interaction and local surface plasmon resonance (LSPR) caused a SEF of aptamer-conjugated Cy3B. In the presence of VEGF, the target-binding interaction of the aptamer led to irreversible detachment of the aptamer from the GNP surface and deactivated surface plasmon enhancement of fluorescent probe (Fig 5B). The aptasensor showed a wide range of linear detection from 25 pg mL^{-1} to 25 µg mL^{-1} for $VEGF_{165}$ with a temporal, thermal, and biological stability. Moreover, authors successfully compared the performance of optimized platform with conventional ELISA.

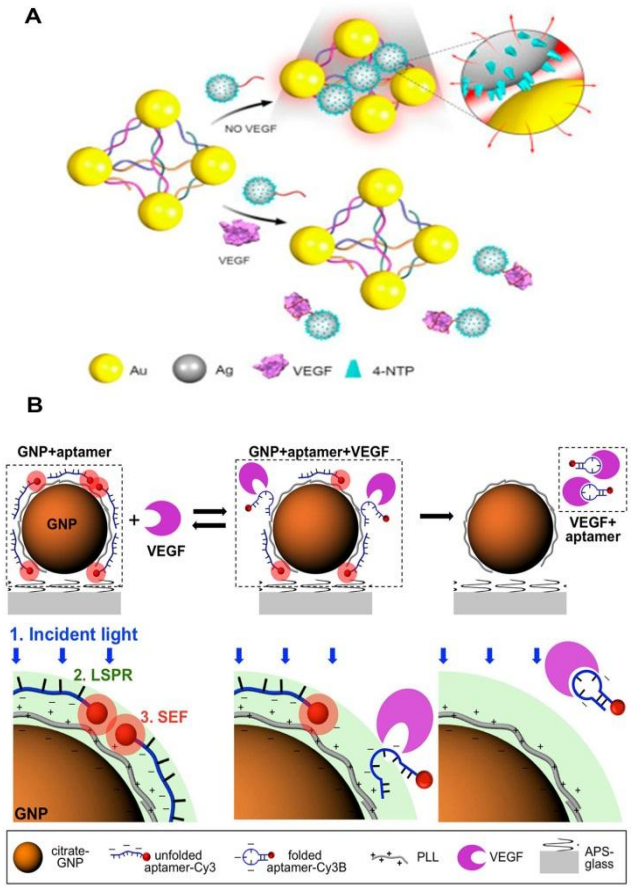

Fig 5. Schematic of (A) An immuno-aptasensor for the detection of VEGF based on SERS technique, (B) Aptamer-based LSPR method for the detection of VEGF. Reprinted with permission from (Zhao et al. 2015) and (Cho et al. 2012); copyright (2017) American Chemical Society.

4. Electrochemical VEGF aptasensors and nano-aptasensors

Typically, electrochemical biosensors operate through detection of output electrical signal generated following specific binding or catalytic reactions of surface modifier biomaterials such as aptamer, enzyme, antibody, or nucleic acids on the surface of a metal or carbon electrode (Luo and Davis 2013; Zhu et al. 2014). Electrochemical biosensors are widely used for biomedical analysis and early detection or monitoring of diseases (Hammond et al. 2016). In recent years, various types of biomolecules with differential electrode stability and selectivity have been used for fabrication of new types of electrochemical biosensors. These advanced electrochemical biosensors focus on some high-throughput methods to improve sensitivity, specificity, and real-time analysis as well as single analyte detection of biomolecules (Danesh et al. 2016; Wang et al. 2017; Zhu et al. 2014). Various electrochemical techniques including amperometric, potentiometric and impedimetric have been used

for biosensing applications and have great potential in the sensing of low- and high-molecular-mass analytes such as protein markers (Hammond et al. 2016; Pan et al. 2017; Wang et al. 2017; Zhu et al. 2014). Different reported electrochemical aptasensors and nano-aptasensors for the detection of VEGF are discussed in this section as summarized in Table 3.

Table 3. Electrochemical aptasensors and nano-aptasensors for detection of VEGF

Detection Method	Strategy	Recognition motif	Limit of detection	Linear range	Type of VEGF	Ref
Aptasensors	Target-induced conformational change/ methylene blue-modified aptamer	DNA aptamer	5 pM	50 pM to 0.15 nM	$VEGF_{165}$	(Zhao et al. 2011)
	Self-assembly of thiolated aptamers on gold-covered surface	DNA aptamer	0.15 ng mL^{-1}	0.15 -100 ng mL^{-1}	-	(Crulhas et al. 2017)
	Cyclic target-induced primer extension	DNA aptamer	0.82 pg mL^{-1}	1 pg mL^{-1} to 1 ng mL^{-1}1	-	(Cheng et al. 2012)
	Capacitive aptamer–antibody based sandwich assay	DNA aptamer	401 pg mL^{-1} at 65 MHz	5 pg mL^{-1} to 1 ng mL^{-1}	$VEGF_{165}$	(Qureshi et al. 2015)
	Aptamer bridged DNA network structure	RNA aptamer	30 fM	100 fM to 10 nM	$VEGF_{165}$	(Da et al. 2018)
	Bound/free separation system	DNA aptamer	-	-	$VEGF_{165}$	(Abe et al. 2012)
	Sandwich method/ pyrroquinoline quinone glucose dehydrogenase (PQQ-GDH)/ enzymatic signal amplification	DNA aptamer	15 nM	15-60 nM	$VEGF_{165}$	(Nonaka et al. 2012)
	Non covalently bonded electroactive label (hemin)	DNA aptamer	1 nM	0 to 80 nM	-	(Lv et al. 2014)
	Electrochemically triggered click reaction	DNA aptamer	6.2 nM	0 to 10 μM	$VEGF_{165}$	(Feng et al. 2016)
	BSA-gold nanoclusters/ionic liquid/ glassy carbon electrode	DNA aptamer	DPV: 0.32 pM EIS: 0.48 pM	DPV: 1–120 pM EIS: 2.5–250 pM	$VEGF_{165}$	(Shamsipur et al. 2015)
	mesoporous carbon-gold nanocomposite modified screen printed electrode	DNA aptamer	1 pg mL^{-1}	10.0 to 300.0 pg mL^{-1}	$VEGF_{165}$	(Amouzadeh Tabrizi et al. 2015)
	rGO-PAMAM / gold nanocomposite / thionine	DNA aptamer	0.7 pM	2.5–320.0 pM	$VEGF_{165}$	(Amouzadeh Tabrizi et al. 2017)
Nanomaterial-based aptasensors	Gold nanostructured graphite screen-printed electrodes (AuNPs/GSPEs)	DNA aptamer	30 nmol L^{-1}	0 and 250 nmol L^{-1}	–	(Ravalli et al. 2015)
	Silicon nanowire, Field-effect transistors (FETs)	RNA aptamer	n-type: 1.04 nM p-type: 104 pM	–	$VEGF_{165}$	(Lee et al. 2009)
	Polypyrrole nanotube, p-type FET	RNA aptamer	400 fM	–	$VEGF_{165}$	(Kwon et al. 2010)
	polypyrrole-converted nitrogen-doped few-layer graphene (PPy-NDFLG), FET	RNA aptamer	100 fM	–	$VEGF_{165}$	(Kwon et al. 2012)
	Ag/Pt bimetallic nanoclusters, Peroxidase-mimicking activity	DNA aptamer	4.6 pmol L^{-1}	6.0 pmol L^{-1} to 20 pmol L^{-1}	$VEGF_{165}$	(Fu et al. 2016)

Zhao et al. (2011) introduced a folding-based electrochemical aptasensor for the detection of VEGF in whole blood. MB-labeled VEGF aptamer was covalently attached to a gold electrode. In the absence of VEGF, the aptamer was partially unfolded thereby no electron transfer and the faradaic current occurred between the terminal MB label and the electrode surface. On the contrary, following VEGF-aptamer specific binding, folding of the aptamer conformationally changed into a stem-loop structure

which led to a reduced distance between MB label and the electrode, resulting in increased electron transfer and faradaic current. The designed aptasensor showed an excellent selectivity, reusability and sensitivity for VEGF detection with a LOD of 5 pM and linear range of 50 pM to 0.15 nM in 50% blood serum.

Crulhas et al. (2017) also reported an electrochemical aptasensor for the detection of released VEGF and prostate-specific antigen (PSA) from cancerous and normal prostate cells. The principle behind this aptasensor was self-assembly of thiolated aptamers to a hairpin conformation on a gold-covered surface using MB as redox label. Briefly, upon binding of VEGF to the aptamer, the hairpin structure of aptamer probe was unfolded which resulted in reduced electron transfer from MB to working electrode surface and consequently change in redox current. LOD of presented aptasensor for VEGF detection was calculated to be 0.15 ng mL^{-1} with a LR of 0.15 -100 ng mL^{-1}.

Cheng et al. (2012) presented an electrochemical aptasensor for protein detection using cyclic target-induced primer extension (CTIPE) and used VEGF as a model protein. In their work, electrochemical signal generation was based on enzyme-amplified method. They immobilized an aptamer hairpin probe on a gold electrode which formed stem-loop structure and blocked primer hybridization. The conformation of hairpin probe changed upon specific VEGF-aptamer interaction and exposed a site for biotinylated-primer hybridization followed by primer extension reaction by the polymerase. Following replication of DNA duplex, VEGF was released and bound with another aptamer hairpin probe, triggering new primer extension in a cyclic process. Finally, streptavidin-alkaline phosphatase (ST-ALP) was attached to the produced biotin-tagged DNA duplex which led to the enzymatic signal enhancement (Fig 6A). The synthesized aptasensor exhibited considerable amplification performance, high sensitivity and good reproducibility without requiring thermal cycling method. The LOD was 0.82 pg mL^{-1} with a linear range from 1 pg mL^{-1} to 1 ng mL^{-1} for VEGF.

Qureshi et al. (2015) designed a label-free electrochemical aptasensor for the detection of VEGF using a capacitive aptamer–antibody based sandwich assay (Fig 6B). Gold interdigitated microelectrode capacitors were functionalized with VEGF aptamer. Aptamer and VEGF antibody-conjugated magnetic beads (MB-Abs) bound to two different epitopes of VEGF and formed a sandwich complex. Non-Faradaic electrochemical impedance spectroscopy (nFIS) was used to sense changes in capacitance against applied AC electrical frequency. The prepared aptasensor exhibited enhanced capacitive signal by 3 to 8-folds and improved sensitivity in the sandwich format. Moreover, the proposed aptasensor achieved a selective and sensitive detection of VEGF in a linear range of 5 pg mL^{-1} to 1 ng mL^{-1}.

Other electrochemical aptasensors were also fabricated for the detection of VEGF using various methods including bound/free separation system (Abe et al. 2012), enzymatic signal amplification (Nonaka et al. 2012), gold electrode/electroactive label (Lv et al. 2014) and electrochemically triggered click reaction (Feng et al. 2016) which have been listed in Table 3.

Utilizing metal nanoparticles and carbon-based nanomaterials for the development of electrochemical sensing platforms is of particular interest (Pan et al. 2017; Taghdisi et al. 2015; Wang et al. 2017; Zhu et al. 2014). Nanomaterials increase output signals of electrochemical biosensors by modifying the surface of electrodes and accelerating electron transfer across the electrode (Luo and Davis 2013; Wang et al. 2017; Zhu et al. 2014). Owing to the unique and extraordinary properties, nanoscale-based electrochemical biosensors have improved sensitivity and lowered limit of detection and also have been regarded as a versatile platform for point-of-care (POC) diagnosis (Hammond et al. 2016; Wu et al. 2016). In this context, a number of electrochemical aptasensors coupled with nanomaterials have been developed for the detection of VEGF. A comparison between the LOD and LR of these reported electrochemical VEGF nano-aptasensors is summarized in Table 3.

Shamsipur et al. (2015) developed a label-free VEGF electrochemical aptasensor, for the first time, based on both differential pulse voltammetry (DPV) "signal off" and electrochemical impedance spectroscopy (EIS) "signal on" methods using a BSA-gold nanoclusters/ionic liquid (BSA-AuNCs/IL) nanocomposite. VEGF aptamer was immobilized on a nanocomposite-modified GCE. Moreover, methylene blue (MB) and $[Fe(CN_6)]^{3-/4-}$ electrochemical probes were used for the "signal off" and "signal on" strategies, respectively. Proportional with increasing concentration of VEGF and binding with the immobilized aptamer, a decrease in current intensity of the DPV of MB probe as well as more mass-transfer limiting of the $[Fe(CN_6)]^{3-/4-}$ probe to the electrode surface in the "signal off" and "signal on" mechanisms were observed, respectively. According to these results, LOD for "signal off" and "signal on" strategies was 0.32 pM and 0.48 pM with a linear range of 1–120 pM and 2.5–250 pM, respectively. In this label-free aptasensing platform, the highly nanoporous structure of AuNCs provided a large surface area for immobilization of VEGF resulting in improved sensitivity and stability.

Another designed label-free electrochemical VEGF aptasensor was reported by Amouzadeh Tabrizi et al. (2015) based on ordered mesoporous carbon–gold nanocomposite ($OMC–Au_{nano}$). Carbon-based screen-printed electrode (SPE) was modified with prepared $OMC–Au_{nano}$ nanocomposite and then, VEGF aptamer was immobilized on it. Cyclic voltammetry (CV) and EIS were used to detect electrochemical changes in interface properties of surface-modified electrodes after VEGF-aptamer interaction. LOD of VEGF was 1.0 pg mL^{-1} with a linear range of 10.0-300.0 pg mL^{-1}. Moreover, the prepared aptasensor successfully detected VEGF in the serum sample of lung cancer patient.

In another study conducted by the same group, an electrochemical bi-aptasensor for the simultaneous detection of cytochrome c (CYC) and VEGF was fabricated (Amouzadeh Tabrizi et al. 2017). They immobilized flavin adenine dinucleotide (FAD) as CYC probe and thionine (Th) as VEGF probe on reduced graphene oxide-poly(amidoamine)/gold nanocomposite (rGO-PAMAM/Aunano) modified dual working screen-printed electrode. Selective interaction of VEGF with rGO-PAMAM-Th/Aunano/anti-aptamer VEGF led to the electrochemical change which was detected by CV and DPV as analytical techniques. The constructed aptasensor exhibited considerable sensitivity, selectivity, and stability with an LOD of 0.7 pM and linear range of 2.5–320.0 pM for VEGF.

In another study, Ravalli et al. (2015) designed an electrochemical VEGF aptasensor based on gold nanostructured graphite screen-printed electrodes (AuNPs/GSPEs). They used two different aptamers; primary thiolated aptamer and secondary biotinylated aptamer, to construct a sandwich-based aptasensor. Moreover, streptavidin-conjugated ALP was used as an enzyme-amplified detection method. First, a sandwich structure was formed on the surface of AuNPs/GSPEs. Then, hydrolysis of the electroinactive 1-naphthyl-phosphate to electroactive 1-naphthol was catalyzed by ALP and finally detected by DPV. This sensing platform showed an LOD of 30 nmol L^{-1} for VEGF.

Lee et al. (2009) developed a label-free aptasensor using anti-VEGF aptamer-modified n-type and p-type silicon (Si) nanowire field-effect transistors (SiNW-FETs). VEGF aptamers were covalently immobilized on the surface of single-crystalline SiNWs. The principle behind of both prepared p-type and n-type SiNW aptasensors was the gating effect of charged VEGF on the surface of Si nanowire channels. Aptamer-VEGF interaction led to change in the charge carriers of the p-type and n-type SiNW-FETs and consequently decreased and increased detection currents, respectively. The reported aptasensor sensitively detected VEGF with a LOD of 1.04 nM and 104 pM for n-type and p-type SiNW-FETs, respectively.

Another FET-based VEGF aptasensor was fabricated using p-type FET and carboxylated polypyrrole nanotubes (CPNTs) was employed as high-performance transducers of this FET system (Kwon et al. 2010). VEGF aptamer was attached to CPNT surface acting as the gate dielectrics of p-type FET

sensor and provided measurable signals following interaction with the aptamer. The LOD of this label-free and real-time CPNTs-aptamer FET biosensor was calculated to be 400 fM. Moreover, this group (Kwon et al. 2012) also designed, for the first time, a FET-type VEGF aptasensor using polypyrrole-converted nitrogen-doped few-layer graphene (PP_y-NDFLG) as a signal transducer. Conducting polymers having heteroatoms in their structures were employed as the carbon source for fabricating doped graphene. They immobilized VEGF aptamer on the PPy-NDFLG and combined them in a FET platform to fabricate a VEGF aptasensor. Addition of VEGF to aptamer led to the change in current output through the FET device. The prepared aptasensor revealed a high sensitivity and rapid response time as well as excellent reusability and mechanical flexibility with a LOD of 100 fM for VEGF.

Recently, Fu et al. (2016) presented a label-free electrochemical VEGF aptasensor based on the intrinsic peroxidase-mimicking activity of DNA-templated Ag/Pt bimetallic nanoclusters. First, a VEGF aptamer was covalently attached to a GCE. The synthesized DNA-Ag/Pt NCs had a ssDNA containing a template DNA and an another 12-mer VEGF aptamer. After addition of VEGF, two aptamers specifically bound to VEGF and formed a VEGF-Ag/Pt NCs complex on the GCE plates. This phenomenon led to the catalytic oxidation of 3,30,5,50-tetramethylbenzidine (TMB) by DNA-Ag/Pt NCs, as signal detection probes, and subsequent increase of current intensity as well as a blue color change in the solution (Fig 6C). The LOD of constructed VEGF aptasensor was 4.6 pmol L^{-1} and LR of 6.0 pmol L^{-1} to 20 pmol L^{-1}.

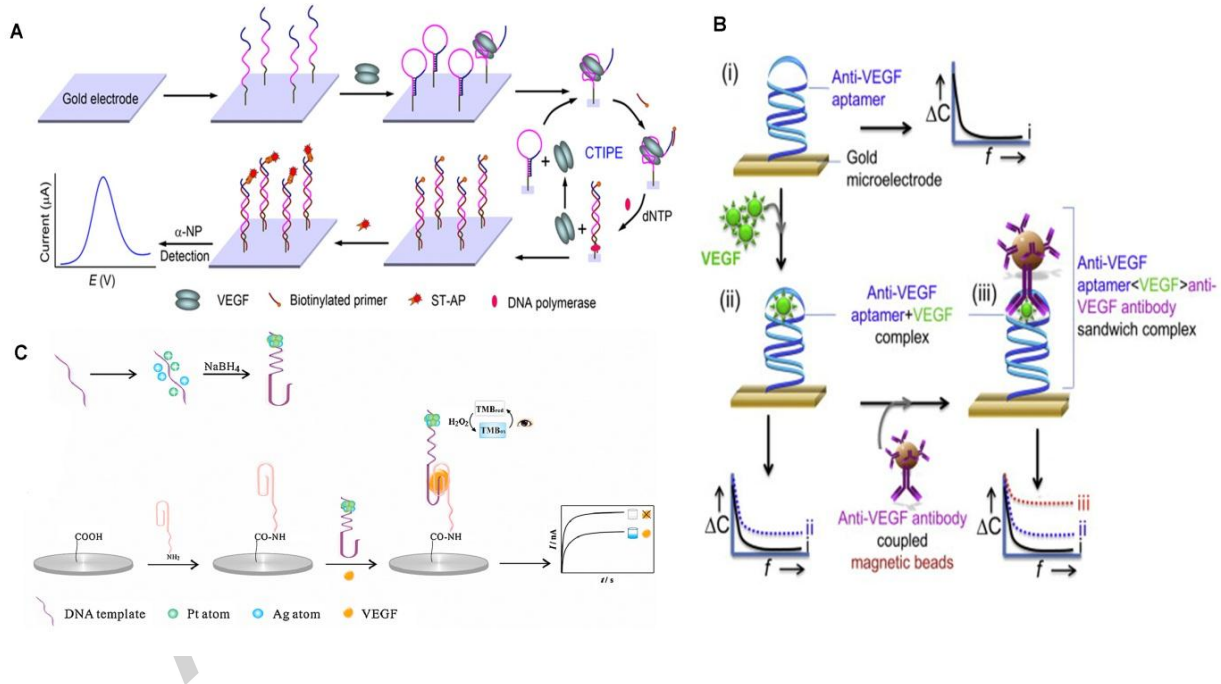

Fig 6. (A) A label-free electrochemical aptasensor for the detection of VEGF using a capacitive aptamer–antibody based sandwich assay, (B) An electrochemical aptasensor for VEGF detection using cyclic target-induced primer extension (CTIPE), (C) A label-free electrochemical VEGF aptasensor based on the intrinsic peroxidase-mimicking activity of DNA-templated Ag/Pt bimetallic nanoclusters. Reprinted with permission from (Qureshi et al. 2015), (Cheng et al. 2012) and (Fu et al. 2016).

Da et al. (2018) have recently synthesized a "signal-off" VEGF photoelectrochemical (PEC) aptasensor using an aptamer-bridged DNA network structure. Graphitic carbon nitride (g-C_3N_4) was utilized as photoactive material. VEGF aptamer-bridged DNA networks were assembled on a g-C3N4 modified GCE which provided a dsDNA platform for the addition of MB in order to expedite electron transfer through its helix structure and enhance the generation of PEC signal. Specific binding of

VEGF to aptamer and formation of aptamer-VEGF complex led to the destruction of DNA network and subsequent release of MB caused a decrease in PEC signal. The developed PEC aptasensor provided an excellent analytical device for the detection of VEGF with an LOD of 30 fM and linear range of 100 fM to 10 nM.

In this review, several optical- and electrochemical-based VEGF aptasensors/nanoaptasensors responding with different manners have been described. Tables 1, 2 and 3 attempted to compare performance of these biosensors. Comparison of advantages and limitations of optical and electrochemical biosensing methods for VEGF detection are summarized in Table 4. The difference between methods can be ascribed to several factors that have affected the aptasensors applicability. These include the type of aptamer (DNA or RNA aptamer), type of sample (buffer/serum), platform compositions, and measurement protocols used. It might be possible to improve the performance of these aptasensors by individually optimizing the factors as well as by using nanomaterials. The comparison between results of different assays demonstrates that the integration of a wide variety of nanomaterials with VEGF aptasensors can improve sensitivity and specificity of aptasensors through sensing of lower concentration of VEGF.

Table 4: Comparison of advantages and limitations of optical and electrochemical methods for VEGF detection

Principle	Advantages	Limitations	Ref.
Optical Aptasensor	- real-time and quantitative - direct and label-free detection - without requirement of amplification and advanced instruments - in colorimetric assay observed with the naked eye - many potential applications in portable systems for colorimetric assay	- commonly expensive - low portability for fluorescence based biosensors - has not been used in clinic - time consuming fabrication	(Jamali et al. 2014; Perumal and Hashim 2014)
Electrochemical Aptasensor	- quantitative - reusable - low volume of sample/reagent is required	- has not been used in clinic - at higher currents, control on the surface of working electrode is limited - false positive results originated from electrolytes	(Jamali et al. 2014; Saei et al. 2013; Zuo et al. 2007)

5. Comparison of VEGF commercial detection kits with the developed aptasensing platforms

Currently, antibody-based assays such as ELISA and Luminex are the only available commercial detection kits for VEGF (Table 5) (Cho et al. 2012; Zhao et al. 2015). Aptamers have recently been used in various VEGF detection platforms as sensing agents. In comparison with aptasensors, antibody-based assays have many shortcomings such as the requirement of large sample volumes, sophisticated instrumentation, expensive antibodies, long incubation times and complicated protocols (Xu et al. 2017; Zhao et al. 2015; Zhu et al. 2017). More importantly, there is a batch to batch variation in affinity of antibodies toward their targets. On the other hand, ELISA has a fairly limited detection range and sensitivity compared to developed aptasensors. Conversely, aptamers have prominent characteristics as mentioned above (Cho et al. 2012; Kopra et al. 2014). Consequently, according to the obtained data from various developed VEGF aptasensors and comparison with available commercial VEGF detection kits, aptasensors show at least equal sensing properties to the commercial VEGF detection kits and unlike the antibody-based detection techniques, can be promising tools for POCT field in the future of clinical diagnostics, and it is currently at the nascent phase.

Table 5: Comparison of analytical performance of numerous VEGF commercial detection kits

Commercial VEGF kit	Assay Sensitivity	Assay Range	Sample Types	Detection Method	Assay Time
Invitrogen™	7.9 pg mL^{-1}	15.6-1,000 pg mL^{-1}	Cell culture supernatants,	Colorimetric	Not reported

			Plasma, Serum	
TiterZyme^{R}	4 pg mL^{-1}	8-2,000 pg mL^{-1}	Cell culture supernatants, Plasma, Serum	2.5 hours
ABIN625107	10 pg mL^{-1}	10-6000 pg mL^{-1}	Cell culture supernatants, Plasma, Serum	Not reported
Quantikine®	9 pg mL^{-1}	15.6 - 1,000 pg mL^{-1}	Cell culture supernatants, Plasma, Serum	4.5 hours
ab100662	< 10 pg mL^{-1}	8.23-6000 pg mL^{-1}	Cell culture supernatants, Plasma, Serum	Not reported
MBS355343	<1 pg mL^{-1}	15.6 -1000 pg mL^{-1}	Human serum, plasma, body fluids, tissue lysates or cell culture supernatants	Not reported

6. Conclusions and future perspectives

In recent years, there has been a growing attention to the use of various types of aptamer-based biosensors for the accurate and rapid identification of VEGF as an important disease biomarker. Unique features of aptamers in comparison to antibodies make these molecular recognition elements versatile choices for developing a very wide range of VEGF sensing platforms. However, despite the successful testing of sensing properties of VEGF aptamers in many sensing procedures, there is still much effort needed to develop applicable and easy-to-use VEGF aptasensors for point-of-care diagnostics. Nowadays, various signal transduction approaches have been developed for the fabrication of aptamer-based VEGF biosensors. The integration of nanomaterials with biosensing method can improve sensitivity and specificity. However, it emphasizes that a general concern regarding bio-safety of nanomaterials might be improved for in vivo and POC detection of VEGF. Generally, two important issues will affect the future of VEGF aptasensors. Firstly, most of the reported aptasensors sense their targets in vitro and as a priority in the future, it is necessary to design aptasensor devices for detecting the presence of VEGF and measuring its concentration in vivo and in the complex clinical samples. Moreover, interference of designed aptamers against VEGF with other protein biomarkers remains a challenge to be solved, because more than one protein biomarker present in most of the human diseases such as cancer. The fabrication of multiple detection platforms integrated with nanomaterials are considered for VEGF to provide information about accurate levels of VEGF compared with other biomarkers. In this regard, one strategy is to design aptasensors arrays for the simultaneous detection of a variety of cancer biomarkers. In summary, together with the recent advancement in the field of aptasensors, drastic developments are expected to happen in the future in parallel with developed on-chip and miniaturized sensing structures such as microfluidic-based aptasensors for the POC detection of analytes with potentially significant applications in clinical diagnostics.

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Conflict of interest

The authors declare no conflict of interest.

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Highlights

- Vascular endothelial growth factor (VEGF) is a cancer-specific protein biomarker.
- This review mainly focuses on recently developed optical and electrochemical aptasensors for VEGF detection.
- This review covers the prominent roles of nanomaterials in the development of VEGF aptasensors.
- Future perspectives and challenges of VEGF aptasensors are discussed briefly.