BioChip J. (2019) 13(3): 203-216
DOI 10.1007/s13206-019-3301-9

Review Article

Electrochemiluminescent Chemosensors for Clinical Applications: A Review

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Received: 14 August, 2019 / Accepted: 02 September, 2019 / Published online: 16 September, 2019

Abstract Economic development has raised concerns about human healthcare and disease prevention from its early stages. In that regard, the detection of biomarkers is crucial for early diagnosis of diseases, and it is an essential tool for managing various health conditions. The clinical diagnostics industry is worth hundreds of billions of dollars and has been expanding. However, the traditional methods for biomarkers detection are high-cost and time-consuming. Also, they usually require highly trained personnel and complex instrumental processes, only providing a centralized medical diagnosis system in large hospitals or specialized facilities. In contrast, a chemosensor is a smart molecular analytical device designed to sense an analyte to generate a detectable signal and to offer direct diagnosis without complex instruments or systems. Moreover, electrochemiluminescence (ECL) possesses distinct advantages such as low-costs, simplicity, and portability. ECL has become a useful technique and has been widely applied in many fields, from basic research to practical applications. Chemosensors coupled with ECL can provide compelling advantages over conventional approaches, such as rapid response time, higher sensitivity, and selectivity. This minireview aims to highlight recent representative studies on ECL-based chemosensors for clinical applications. It provides a general overview of the design and structure of ECL-based chemosensors, and also covers the general problems and challenges. The presented content may prove to be useful for discovering new sensor concepts or extension of existing biomarker detection strategies.

Keywords: Electrochemiluminescence, chemosensors, clinical applications, luminophore, coreactant

Introduction

A chemosensor is a chemical molecule composed of two subparts: a recognition unit for specific binding to a molecular analyte and a signaling unit that reports the binding event in the form of a detectable signal[1,2]. Unlike most bioanalytical methods which exploit biological receptors such as peptides, proteins and nucleic acids (e.g. antibodies, aptamers, etc.) as their recognition element, the chemosensor relies on recognition elements of abiotic origin[3,4]. If the binding event between the recognition unit and the analyte is of irreversible nature (i.e. formation of covalent bonds), the sensor is termed a chemodosimeter. The fluorescent chemosensor for the detection of aluminum ion developed in 1867 by Friedrich Goppelsroder can be considered to be the first chemosensor in history, which not only pioneered the field of chemosensing in particular but also modern analytical chemistry in general. Subsequently, substantial progress was made in developing more chemosensors, primarily for metal ion sensing, by Anthony W. Czarnik[5-7], A. Prasanna de Silva,[8-10] Roger Tsien[11,12], Lynn Sousa[13] and others. At the present, applications of chemosensors can be found in various fields and industries, such as clinical analysis[14-16], food analy-

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sis[17-19] and environmental analysis[20-22]. Depending on the response produced by the signaling element, chemosensors can be further categorized into different types, including fluorescent chemosensors, chemiluminescent chemosensors, electrochemiluminescent (ECL) chemosensors, colorimetric chemosensors, electrochemical chemosensors, surface plasmon resonance chemosensors, quartz crystal microbalance chemosensors or a combination of more than one signaling method (i.e. multi-signal chemosensors)[23]. Nevertheless, the majority of chemosensing research has been focused on fluorescent chemosensors, due to their advantages including relative simplicity, good selectivity and high sensitivity[24-27]. However, the dependence of fluorescent chemosensors on an excitation light source limits their clinical point-of-care diagnostic application. Furthermore, fluorescent chemosensors also suffer from background noises (i.e. auto-fluorescence, scattered light) interfering with the desired signal and reducing the signal-to-noise ratio[28].

Electrochemiluminescence (also known as electrogenerated chemiluminescence) is a type of luminescence involving light emission upon relaxation of excited states produced from highly energetic electron-transfer reactions between electrochemically generated species at an electrode surface[29]. Since the first detailed reports on ECL in the 1960s[30-32], ECL has grown to become a crucial tool for analysis across various fields such as immunosensing, aptasensing, and chemosensing. The significance of ECL is due to its unique features which combine the advantages of photoluminescence and electrochemical methods, separating ECL from other types of luminescence. Firstly, ECL does not require the use of a light source as does photoluminescence, thus simplifying the optical instrument setup and reducing luminescent interferences. And secondly, the signaling event in ECL is triggered by an applied potential, providing ECL with better spatial and temporal control as well as higher selectivity compared to chemiluminescence[33]. A comparison between photoluminescence, electrochemiluminescence and electrochemical methods is given in Table 1. The excited states which emit light in ECL are produced from reactive intermediates formed upon electrochemical oxidation/reduction of stable, ECL-active species. Thus, ECL can be considered a form of chemiluminescence in which the reactants are electrochemically produced on the electrode surface. Luminophores employed in ECL-chemosensors belong to one of the three categories: organic luminophores (e.g. BODIPY dyes, thiophene-based molecules)[34,35]; inorganic luminophores (e.g. tris(2,2'-bipyridine) ruthenium(II) complexes, cyclometalated iridium complexes)[36,37]; or nanomaterial-based luminophores (e.g. gold nanoclusters, C_3N_4 quantum dots)[38,39].

ECL can be produced through two pathways: annihilation ECL or coreactant ECL. Annihilation ECL, or radical ion annihilation ECL, is the ECL pathway in which an oxidized form and a reduced form of the ECL-active species are sequentially produced at the surface of the electrode by rapidly alternating the potential between two values and subsequently undergo an annihilation reaction to generate the emissive excited state. The excited state $^1A^*$ in this instance is a singlet excited state. The system that is capable to directly produce a singlet excited state is called an energy-sufficient system or S-route (singlet route) owing to the fact that the energy provided by the ion radicals through the annihilation reaction is sufficient to populate the singlet excited state. The annihilation process for an energy-sufficient system is described by reactions 1-4, where A and D can represent the same, or different ECL-active species. $Ru(bpy)^{2+}$ is perhaps the most well-known example of an energy sufficient system[40].

Table 1. A comparison between electrochemiluminescence, photoluminescence and electrochemical methods[40,41,98].

Parameters	Electrochemiluminescence	Photoluminescence	Electrochemical methods
Instrumentation	Compact and inexpensive	Bulky and costly	Compact and inexpensive
Sensitivity	High (Electrochemically induced emission)	Low (Photochemically induced emission)	Medium (Electrochemically induced signal)
Matrix effects	Low (No light source is used)	High (Unselective photoexcitation)	Medium (Electrical interferences)
Temporal and spatial control	Controlled by the applied potential	Controlled by the light source	Controlled by the applied potential or current
Portability	Portable	Non-portable	Portable

$$D - e^- \rightarrow D^{+\bullet} \quad (1)$$

$$A + e^- \rightarrow A^{-\bullet} \quad (2)$$

$$A^{-\bullet} + D^{+\bullet} \rightarrow {}^1A^* + D \quad (3)$$

$${}^1A^* \rightarrow A + h\nu \quad (4)$$

In contrast, if the energy is not large enough to populate the singlet excited state, triplet excited states can be populated instead. The triplet excited states populated in this energy deficient system then undergo a triplet-triplet annihilation reaction in which the energy from two electron transfer reactions is pooled into the production of a singlet excited state[41]. The process is hence called the T-route or triplet route and is described by reactions 5-9.

$$D - e^- \rightarrow D^{+\bullet} \quad (5)$$

$$A + e^- \rightarrow A^{-\bullet} \quad (6)$$

$$A^{-\bullet} + D^{+\bullet} \rightarrow {}^3A^* + D \quad (7)$$

$${}^3A^* + {}^3A^* \rightarrow {}^1A^* + A \quad (8)$$

$${}^1A^* \rightarrow A + h\nu \quad (9)$$

In the case in which the cathodic radical or anodic radical of the ECL-active species is unstable or cannot be generated due to the extreme reduction or oxidation potentials required, or if one wishes to produce ECL at a single potential step, a coreactant can be incorporated into the system. A coreactant is a species which produces a strong oxidant upon reduction (hence called an oxidative-reductive coreactant, such as oxalate) or a strong reductant upon oxidation (hence called a reductive-oxidative coreactant, such as benzoyl peroxide). The reaction process for the two types of coreactant is described by reactions 10-19. More coreactants are being developed, in part to provide for ECL sensors, which mainly utilize coreactant ECL.

Oxidative-reductive coreactant:

$$A - e^- \rightarrow A^{+\bullet} \quad (10)$$

$$C_2O_4^{2-} - e^- \rightarrow C_2O_4^{-\bullet} \quad (11)$$

$$C_2O_4^{-\bullet} \rightarrow CO_2^{-\bullet} + CO_2 \quad (12)$$

$$CO_2^{-\bullet} + A^{+\bullet} \rightarrow {}^1A^* + CO_2 \quad (13)$$

$${}^1A^* \rightarrow A + h\nu \quad (14)$$

Reductive-oxidative coreactant:

$$A + e^- \rightarrow A^{-\bullet} \quad (15)$$

$$(C_6H_5CO)_2O_2 + e^- \rightarrow (C_6H_5CO)_2O_2^{-\bullet} \quad (16)$$

$$(C_6H_5CO)_2O_2^{-\bullet} \rightarrow C_6H_5CO_2^- + C_6H_5CO_2^{\bullet} \quad (17)$$

$$C_6H_5CO_2^{\bullet} + A^{-\bullet} \rightarrow C_6H_5CO_2^- + {}^1A^* \quad (18)$$

$${}^1A^* \rightarrow A + h\nu \quad (19)$$

Biomarkers are "a characteristic that is objectively measured and evaluated as an indicator of normal biological processes, pathogenic processes, or pharmacologic responses to a therapeutic intervention"[42]. Biomarkers have risen to become an important tool in disease detection and treatment follow-up, especially in the detection of serious health conditions such as cancer, Alzheimer's disease and cardiovascular diseases in early phases[43-47]. The detection of biomarkers remains a challenging task due to them existing at exceedingly low concentrations in complex matrices that are human bodily fluids[43]. Recently, ECL-chemosensing has emerged as a promising method for biomarker detection because of its low costs and high sensitivity and selectivity.

Despite its relatively short history, there have been many excellent comprehensive reviews on ECL sensing and ECL in general[48-53]. However, a review dedicated to the application of ECL chemosensors to the important, rapidly-growing field of clinical analysis is still absent. In this mini review, we focus on ECL chemosensors aimed at detecting clinically important biomarkers and discuss the detection mechanism strategies employed therein as well as the problems and challenges faced by ECL chemosensors.

ECL Chemosensors for Clinical Applications

A typical setup for ECL detection is small-scale and compact, consisting of a sensing platform (i.e. the dark box) in which the electrodes and sample solution are placed, a detector (e.g. a PMT) for ECL signal detection, a potentiostat to control and measure electrochemical parameters and a PC to display and process the signal obtained (Figure 1A). In the sample solution, whose volume is small and can be in the range of a hundred microliters, the ECL turn-on probe reacts selectively with the analyte, turning the probe from an ECL-inactive or weakly ECL-active molecule into a strongly ECL-active product. Measurements are performed by injecting the sample solution onto the electrode surface. Upon the application of an appropriate potential, the product and coreactant undergo electrochemical oxidation-reduction reactions at the electrode surface to generate radical ions, which produce the excited species that emits light via electron-transfer reactions. On the contrary,

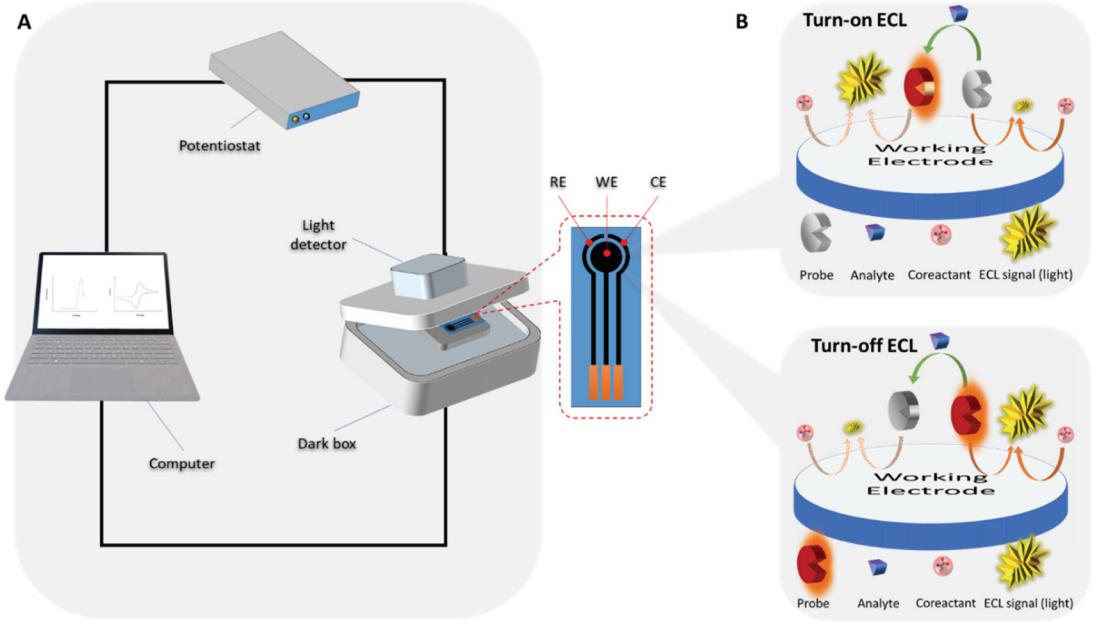

Figure 1. (A) Schematic diagram of an ECL system and (B) Detection mechanisms of ECL chemosensors.

an ECL turn-off probe is strongly ECL-active and will become ECL-inactive after reacting with the analyte (Figure 1B).

Homocysteine

Small-molecular biothiols play crucial roles in biological systems and abnormal levels of these biothiols are strongly related to various diseases. Therefore, monitoring of biothiol concentrations is of great importance in medical diagnostics. Among biothiols, homocysteine (Hcy) is an essential amino acid in human blood. An elevated level of Hcy is a risk factor for cardiovascular diseases and Alzheimer's disease (normal Hcy range in the blood is 5 – 15 μM)[54,55]. Previous approaches utilized enzymes for selective detection of Hcy. However, this strategy requires high costs, time-consuming sample preparation, hindering their use in large-scale applications[56]. Kim et al. achieved a breakthrough by designing Probe **1** based on a cyclometalated iridium(III) complex[37]. Probe **1** incorporates a more strongly electron-withdrawing isoquinoline unit in the main ligand instead of pyridine to stabilize its the lowest-unoccupied molecular-orbital (LUMO), enabling electron transfer from the coreactant TPrA radical, which was confirmed experimentally and by density function theory calculations (Figure 2A). As a result, a turn-on ECL signal is achieved and is in good agreement with PL results. Probe 1 responded linearly to a range of 0 to 40 μM of Hcy concentrations, which covers the normal range of Hcy levels in the blood (Figure 2B). In the competitive binding assay, Probe 1 exhibited no significant change upon the addition of a 200-fold excess of interferences and a remarkable increase upon the addition of 100 equivalents of Hcy (Figure 2C). Probe 1 also shows selective behavior over Cys, which is a strong interference due to its similar structure to Hcy (Figure 2D).

One of the major limitations of reported chemosensors in the literature is that most studies were only conducted in buffer solution environment or deproteinized serum, which necessitate long-time pretreatment. For the first time, Stewart et al. offered a method to directly detect hyperhomocysteinemia in the blood based on the quenching effect of cathodic ECL of near-infrared quantum dots upon addition of Hcy. The quenching rate in blood is significantly lower compared to that in PBS buffer, which is possibly caused by electrostatic interactions between Hcy and biomolecules in blood. However, a linear relationship between the ECL signal and Hcy concentration was obtained in blood and not in PBS. Although the selectivity of this approach needs further optimization, it will inspire other researchers to develop novel sensors that do not require sample pretreatment[57].

Cysteine

Cysteine is also an important biothiol that is involved in different biosynthetic and metabolic processes. An

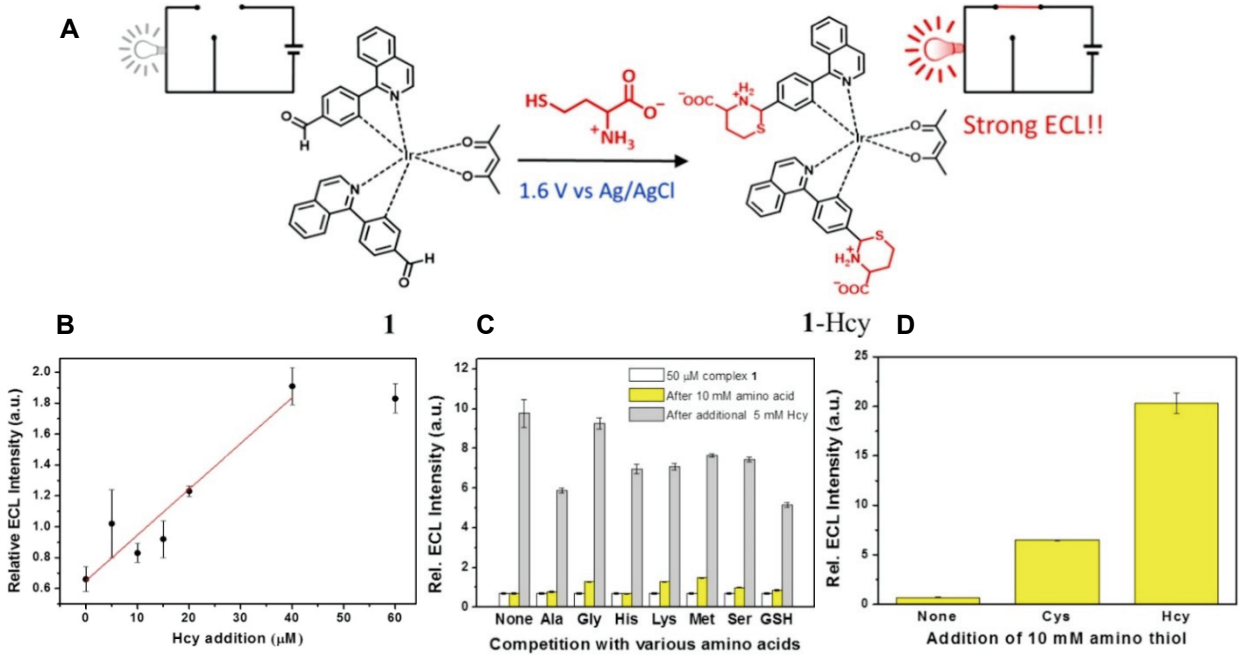

Figure 2. (A) Homocysteine detection by probe 1. (B) Calibration curve of 0.1μM of probe 1 upon addition of Hcy. (C) Competitive ECL binding assays of 5mM Hcy and 10mM various amino acids. (D) Comparison of ECL signal of 10mM Hcy and 10mM of Cys. Reprinted from Ref.[37]. Copyright (2017), with permission from Elsevier.

increase or decrease in the level of cysteine (Cys) is an indicator of a range of different health problems such as brain ischemia, slowed growth, Alzheimer's disease or osteoporosis[58-60]. Xie et al. investigated the ECL properties of an organic fluorescent dye-based probe, which is designed to contain a fluorescein signaling unit and a nitroolefin binding site to interact with Cys. Cys is detected using Michael reaction, the binding adduct exhibits intense ECL signal in the presence of potassium persulfate as a reductive oxidation coreactant. This work shows a linear range of Cys from 10^{-9} to 10^{-8} M and an excellent LOD of 4.2 x 10^{-10} M. However, selectivity tests with other biothiols that possess similar structure to cysteine (i.e. homocysteine, glutathione) were not included[61]. Recently, a cyclometalated iridium(III) complex-based sensor was developed by Kim et al. which can selectively detect Cys over structurally similar compounds: Hcy and GSH. The sensing mechanism is based on phosphorescence enhancement and ECL quenching in the blue-shifted region[62].

Very recently, nanomaterials have emerged to be used as the signaling unit for chemosensors. Zhu et al. demonstrated *in situ* sulfur-doped graphitic carbon nitride nanosheets (S-g-C_3N_4 NSs) for the detection of Cys in human serum using the ECL method. The ECL onset potential of S-g-C_3N_4 is shifted positively compared to g-C_3N_4 (without doped sulfur), likely because the presence of the doped sulfur atoms helps lower the potential barrier, leading to more free S-g-C_3N_4 $NSs^{\bullet-}$ radicals and thus enhancing the electron-hole recombination efficiency. The ECL intensity of the off-on S-g-C_3N_4 NSs sensor is quenched by Cu^{2+} and is returned upon the addition of Cys due to Cu^{2+} and Cys having a higher coordination ability compared to Cu^{2+} and S-g-C_3N_4 NSs. This technique shows a wide linear curve from 20 nM to 0.2 mM with a LOD of 5 nM (S/N = 3)[63].

Glutathione

In addition to Hcy and Cys, glutathione (GSH) is another important biothiols. GSH is a key endogenous antioxidant, the ratio of GSH (reduced form) and GSSG (oxidized form) is a marker of oxidative stress status. Abnormally high or low concentrations of GSH are closely linked to serious diseases associated with cancer, heart problems, aging and HIV[64,65]. Therefore, developing a portable and reliable method to quantify GSH levels is necessary. Recently, a new ECL platform was reported by Niu et al. The technique is based on the coreactant ECL of C-dots and $S_2O_8^{2-}$, amplified by $Fe(CN)_6^{3-/4-}$ redox couple, which can act as the hole-injector to convert more C-dots to C-$dot^{\bullet+}$ to participate in the annihilation reaction. The system is used to detect GSH as the sulfhydryl groups

of GSH can react with $SO_4^{\bullet-}$, effectively quenching the ECL signal. Although the calibration curve is narrow (0.1-1 μM), the developed method demonstrated the potential application of C-dots in ECL chemosensors[66].

Dopamine

Dopamine (DA) is a neurotransmitter in the central and peripheral nervous system, responsible for essential neuronal functions including emotion, movement, behavior, cognition, attention, learning, and memory[67]. Abnormal dopamine levels are associated with neurological disorders such as Alzheimer's and Parkinson's diseases[68,69]. Normal DA level is notably low (0.01-1 μM) in comparison with other interferences that exist in too high concentrations (i.e. normal range of ascorbic acid is 0.1-0.6mM). As a result, tremendous efforts have been made to develop highly sensitive, selective and reliable sensors for DA[67,70].

Many authors applied new classes of luminophores to modified electrodes to detect DA based on the quenching effect via resonance energy transfer (RET) or energy transfer (ET). Wang et al. precisely doped mono-Cu^+ into Cd-In-S super-tetrahedral chalcogenide nanoclusters (Cu@CdInS NCs) to introduce new Cu^+/Cu^{2+} energy states to the system, enhancing its ECL signal. DA can interact with the negatively charged Cu@CdInS NCs and be oxidized by NC^*, effectively quenching its ECL emission. Therefore, this material is immobilized on GCE via a dropcasting method to fabricate a DA sensor. The method shows a linear range from 0.5 μM to 100 μM with a LOD of 0.355 μM[71]. Another strategy was developed based on dual-stabilizers-capped CdSe quantum dots, which achieves a wide linear range of DA (from 10 nM to 3 μM) with a LOD of 3 nM. However, the fabrication process of this sensor is rather complex[72].

Recently, ECL emission of organic nanoparticles received much attention due to their environment-friendliness, non-toxicity, diverse structures and flexible synthesis[73]. Feng et al. successfully synthesized silole-containing polymer nanodot (SCP dots) and proved their potential application in detecting quencher-related analytes. SCP dots were prepared by nano-precipitation method and exhibited a 100-fold enhancement of their ECL signal compared to SCP (cast on a modified GCE). SCP dots showed a linear range of dopamine levels from 0.05 to 10 μM with a detection limit of 50 nM[74]. This study shows the feasibility of using organic nanoparticles in ECL systems and ECL sensing as a substitute for toxic semiconductor quantum dots. However, challenges remain in controlling the particle size and shape of organic nanoparticles to improve their low diffusion coefficient and relatively weak ECL intensity[73,75].

Tryptophan

Tryptophan (Trp) is a vital amino acid in the human body, having important functions in different biological processes. Trp is a precursor for serotonin (a neurotransmitter) and melatonin (a neurohormone). Trp is also a common antioxidant in the food industry and a biomarker in the pharmaceutical industry. Abnormalities in Trp levels could lead to serious health problems such as albinism, alkaptonuria, depression, etc[76-78]. Chen et al. were successful in applying the ECL technique to monitor Trp concentrations. The probe is an iridium (III) complex-based lab-on-a-molecule to detect multiple analytes (photoluminescence and UV-Vis for Cys/His and ECL for Trp). The concept relies on the electrochemical oxidation of indole which decreases the formation of electrogenerated excited states, thus quenching ECL emission. The developed method shows great selectivity over other interferences, but only achieved a non-linear correlation. Nevertheless, the design concept holds promising potential, especially for dealing with multianalyte detection in complex environments[79].

Histidine

Histidine (His) an important α-amino acid which is involved in various important cellular functions, metabolism, and cell regulations. Abnormal levels of His are connected with various health problems such as pulmonary disease, chronic kidney disease, and psychological disorders[80,81]. Zhou et al. demonstrated the detection of His by synthesizing an iridium(III) solvent complex with acetonitrile ligand ($Ir(ppy)_2NCCH_3$) (Probe 2, Figure 3A). The reaction mechanism is attributed to the replacement of the solvent ligands by histidine. ECL intensity increased linearly upon addition of His from 0 to 46 μM with a LOD of 0.25 μM as shown in Figure 3B and 3C. The specificity of Probe 2 was also confirmed by testing with various amino acids such as Met, Gly, Lys, Phe, Val, Trp, and Leu (Figure 3D)[82].

Hydrogen Sulfide

Hydrogen sulfide is the third member of the gasotransmitter family and is believed to function as a neuromodulator in the brain. It is suggested that low levels of H_2S may cause the dysfunction of cerebral microvasculature that results in Alzheimer's disease[83,84].

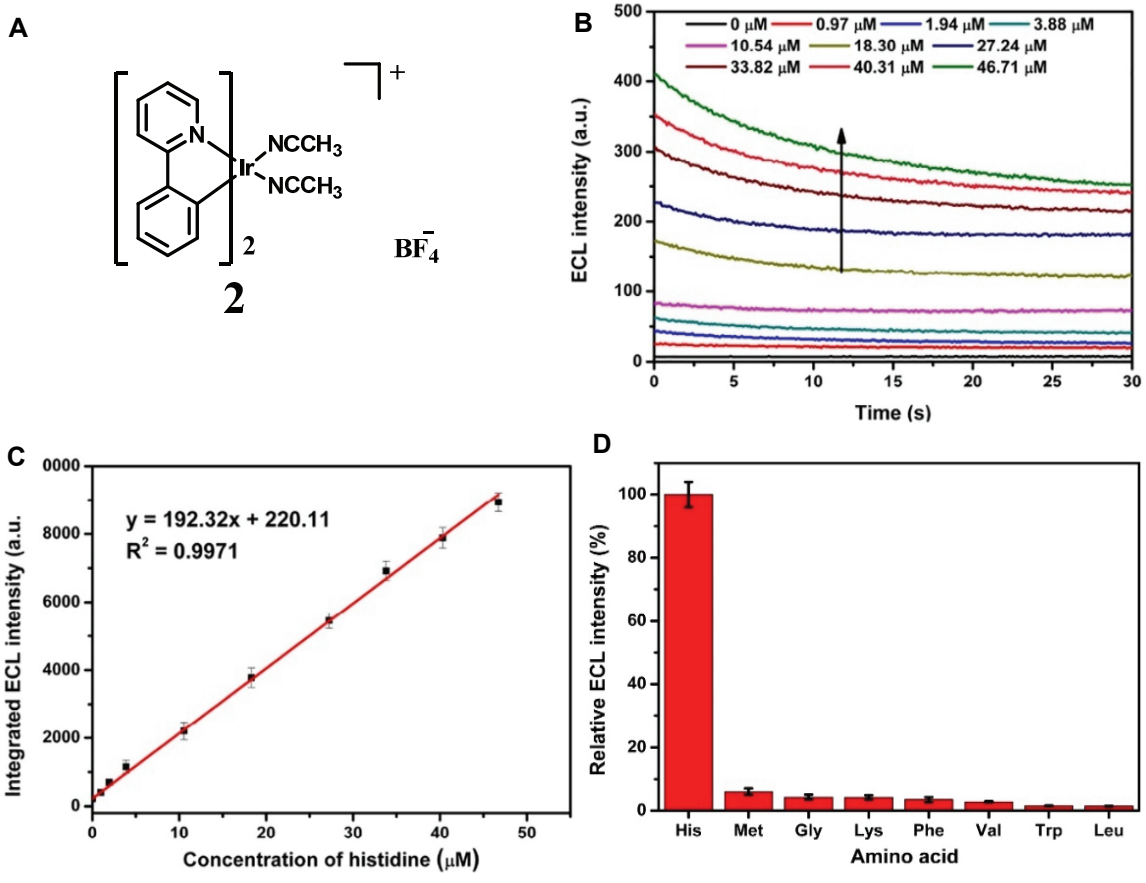

Figure 3. (A) The chemical structure of Probe 2. (B) ECL intensity increased upon addition of histidine. (C) Calibration curve of integrated ECL intensity as a function of histidine concentration. (D) The specificity of Probe 2 to amino acids. Reprinted from Ref.[82]. Copyright (2017), with permission from Elsevier.

Park et al. suggested a new design of a probe for detecting H_2S by attaching o-(Azidomethyl)benzoate (AzMB) ester groups as the receptor unit on the main ligands (Probe 3). Sulfide selectively reacts with the ester groups of Probe 3, causing a structural change to the probe that results in an unfavorable electron transfer of tripropylamine radicals, significantly quenching the ECL intensity of Probe 3 (Figure 4A)[85]. Upon addition of NaHS, ECL emission was quenched and decreased linearly over the range of 40 to 140 μM (Figure 4B). The LOD achieved using ECL method is markedly lower (11 nM) compared to PL (photoluminescence) method. In addition, this probe has excellent selectivity across various anions, except for iodide ions because iodide adsorption on the electrode can hinder the oxidation process needed for ECL (Figure 4C)[86].

Kim et al. designed an off-on probe by using dinitrophenyl (DNP) group as both the quencher unit and the receptor unit. The photo-induced electron transfer (PET) dinitrophenyl (DNP) moiety is either attached only on the ancillary ligand or on both the main and ancillary ligand. Both of the probes exhibit an increase of ECL signal in the presence of S^{2-}. However, the absolute ECL intensity of $(DND)_2$-Probe is substantially lower than that of DND-Probe, leading to a lower sensitivity, which can be rationalized by the presence of the DND moiety on the main ligand, which will form a hydroxyl group after reaction, destabilizing the HOMO level, thus impeding the electron transfer from the coreactant (tripropylamine). On the other hand, one more DND group leads to higher selectivity for $(DND)_2$-Probe[87].

Hypochlorous Acid (HOCl)

HOCl is an essential reactive oxygen species (ROS), produced from the reaction of hydrogen peroxide (H_2O_2) and chloride ions (Cl^-) catalyzed by *myeloperoxidase* enzyme (MPO). Properly controlled production of HOCl is necessary for the human body to respond to invading bacteria and pathogens. However,

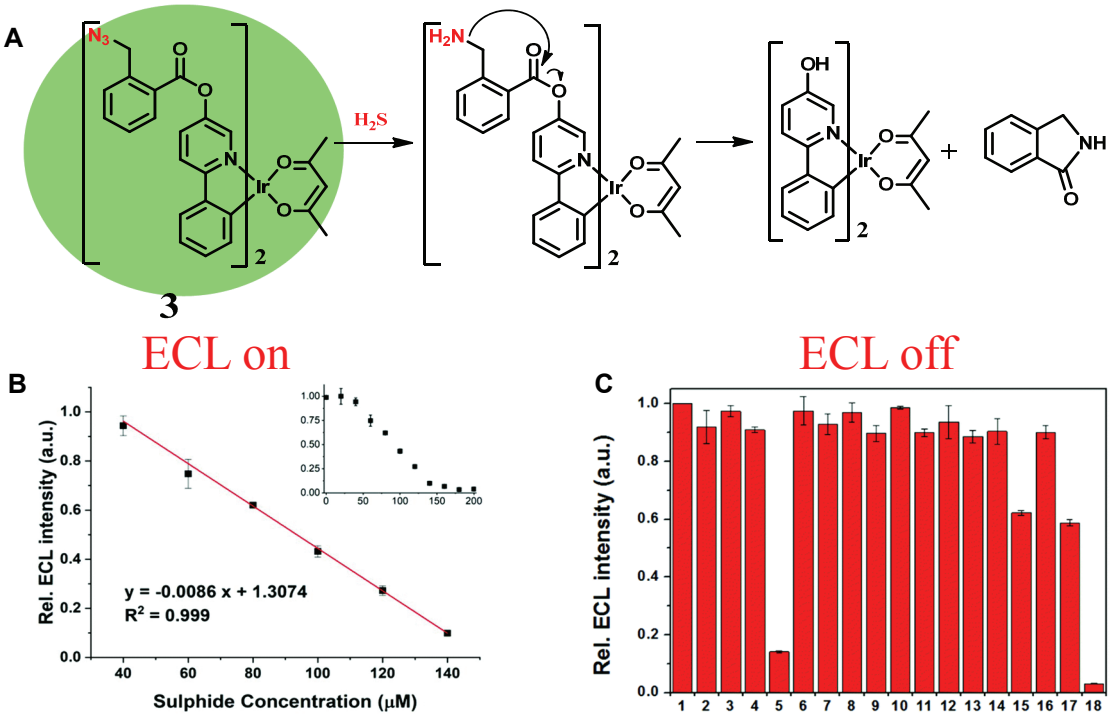

Figure 4. (A) H_2S detection by Probe 3. (B) ECL signal response of Probe 3 (10μM) upon addition of sulfide in CH_3CN/DMSO (5:1 v/v, 10 mM TPA, and 0.1M $TBAPF_6$ as supporting electrolyte). (C) Selectivity tests of probe 3 with 200μM of anions ((1) Probe 3 only (2) F^-(3) Cl^- (4) Br^- (5) I^- (6) NO_3^- (7) NO_2^- (8) N_3^- (9) SO_4^{2-} (10) SO_3^{2-} (11) $S_2O_3^{2-}$ (12) $S_2O_4^{2-}$ (13) $S_2O_5^{2-}$ (14) SCN^- (15) Cys (16) Hcy (17) GSH (18) HS^-. Reprinted from Ref.[85] with permission from The Royal Society of Chemistry.

the overproduction of HOCl is related to diseases such as atherosclerosis, cancer, cardiovascular diseases, and rheumatoid arthritis[34,88]. Therefore it is necessary to monitor HOCl levels in the human body. Cao et al., for the first time, reported a ruthenium(II) complex-based chemosensor incorporating a quencher moiety (ferrocenyl moiety) with bipyridine- ruthenium(II) complex core through a reactive linker (hydrazine). Upon reaction with HOCl, the ferrocenyl quencher moiety will be released, turning on the luminescence signal of the bipyridine-ruthenium(II) complex. ECL signal is observed to increase as HOCl concentration increases in 25 mM PBS-ethanol (3:1, v/v) mixed solvent with 10 mM TPrA as coreactant[89]. Another chemosensor with a similar concept in which iridium is the metal core instead of ruthenium was also reported by the same research group[90].

Pyrophosphate ($P_2O_7^{4-}$: PPi)

Pyrophosphate is a biologically important anion, having roles in processes such as DNA and RNA polymerization, adenosine triphosphate hydrolysis, cyclic adenosine monophosphate synthesis, etc. Abnormal PPi levels are a marker of numerous diseases such as cancer, arthritis or kidney stones[91,92]. Thus, quantification of PPi concentrations is of crucial importance for disease prevention. Shin et al. reported a novel probe with boron dipyrromethene as the signaling unit and a phenoxo-bridged bis(Zn^{2+}–dipicolylamine) as the receptor unit (Probe 4). The sensor can detect PPi based on an on-off mechanism similar to photo-induced electron transfer (PET) in which PPi forms a bridge with two Zn^{2+} cations, increasing the electron density on the nitrogen atoms of the two dipicolylamine moieties, allowing for electron donation from the HOMO of the (Probe 4-PPi) complex to the ground state of the excited boron dipyrromethene moiety, therefore effectively quenching the ECL emission (Figure 5A). Probe 4 shows a linear calibration curve from 6.6 to 13.3 μM with a LOD of 4.0 μM (Figure 5B, 5C). A competitive binding assay demonstrated high selectivity over other anions such as iodide, chloride or phosphate-containing anions such as adenosine-5'-triphosphate (ATP), adenosine diphosphate (ADP), and adenosine monophosphate (AMP) (Figure 5D, 5E)[93].

Xu et al. reported another sensor for pyrophosphate, also employing graphite carbon nitride nanosheets (g-C_3N_4 NSs). The g-C_3N_4 NSs show intense

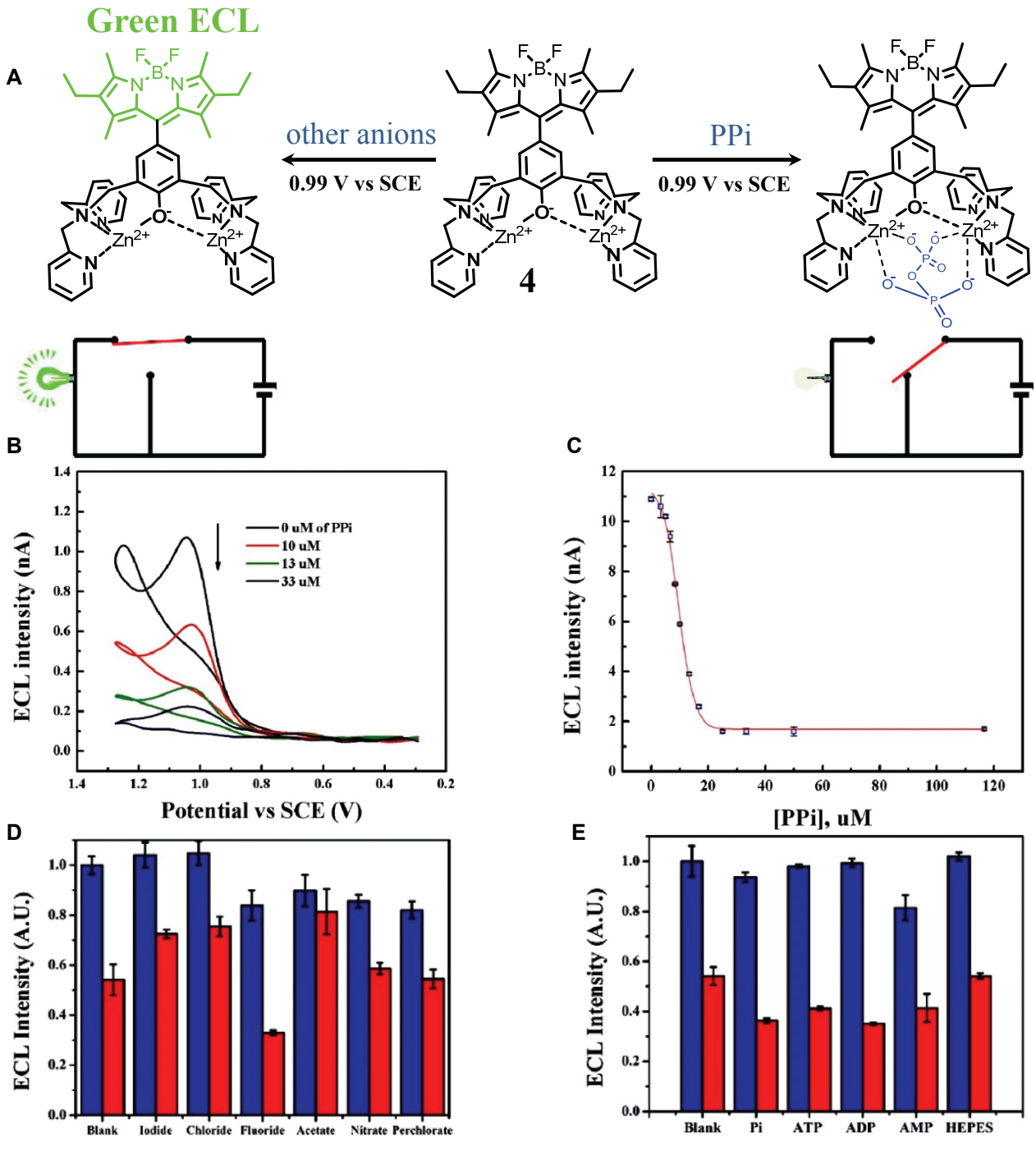

Figure 5. (A) Pyrophosphate detection by Probe 4. (B, C) ECL intensity of Probe 4 is quenched upon addition of PPi in MeCN. (D, E) Competitive assays upon addition of 10μM PPi to 10μM Probe 4 in the presence of (D) 100μM anions and (E) 10μM Pi-containing anions. Reprinted with permission from Ref.[93]. Copyright (2010) American Chemical Society.

ECL intensity, which is quenched in the presence of Cu^{2+} via photo-induced electron transfer (PET). The ECL signal of the system can be recovered upon the addition of PPi anion due to the strong affinity of Cu^{2+} with PPi. Cathodic ECL conducted using 10mM $S_2O_8^{2-}$ as co-reactant can detect PPi in the linear range of 2.0 nM to 800 nM with a LOD of 75 pM. The approach also shows good selectivity over other interferences and can quantify PPi in synovial fluid successfully[94].

Vitamins

Vitamin B_2 (also known as riboflavin) is an essential element required in various cellular processes, such as the metabolism of fats, carbohydrates, and proteins. Vitamin B_2 is a water-soluble vitamin that cannot be produced by the human body and can only be consumed through food or medicine supplements[95]. The variation of vitamin B_2 levels needs to be monitored to diagnose health problems such as skin rashes[96]. Wang et al. successfully fabricated a sensor based on graphitic carbon nitride quantum dots (g-CNQDs). ECL signal is emitted in the presence of $K_2S_2O_8$ as a coreactant and can be quenched via resonance energy transfer upon the addition of riboflavin (vitamin B_2). In this system, g-CNQDs act as the donor and riboflavin (the analyte) as the receptor. The sensor exhibits a linear range from 0.02 μM to 11 μM with a LOD of 0.63 nM with good selectivity. The approach also proves its potential practical application with high recovery in the human serum[97].

Conclusion and Prospects

While fluorescent chemosensors or colorimetric chemosensors have been in existence for a long time, ECL chemosensors have a rather short history. However, one can still witness the rapid expansion of this particular field, not only in terms of chemosensing design concepts but also the various analytes detected using ECL chemosensors. In this review, we introduced outstanding studies on electrochemiluminescent chemosensors for clinical applications. ECL chemosensors have attracted much attention due to the high demand for novel, cutting-edge, portable and reliable sensors with high sensitivity and selectivity to detect more and more analytes in human healthcare. In the future, electrochemiluminescent chemosensors for clinical applications still have broad space for development. As novel concepts need to be developed to design new sensors that can detect more analytes. This is an important mission as more evidence for the correlations between biomarkers and biological processes are being discovered. It is also crucial to develop sensors that can accurately quantify analytes and overcome reproducibility and selectivity issues caused by various interferences in highly complex environments such as human serum or blood.

Acknowledgements This work was supported by the Technology Innovation Program (10077599 and 10077648) funded by the Ministry of Trade, Industry & Energy, Korea. It was also supported by the Basic Science Research Program (NRF-2017R1D1A1B03028668) through the National Research Foundation of Korea (NRF) funded by the Ministry of Education (MOE), and by Original Technology Research Program for Brain Science (NRF-2017M3A9D8029943) funded by Ministry of Science, ICT & Future Planning (MSIP).

Conflict of Interests The authors declare no competing financial interests.

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