Review

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Bioanalysis

A review of electrogenerated chemiluminescent biosensors for assays in biological matrices

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Electrogenerated chemiluminescence (ECL) is the production of light via electron transfer reactions between electrochemically produced reagents. ECL-based biosensors use specific biological interactions to recognize an analyte and produce a luminescent signal. Biosensors fabricated with novel biorecognition species have increased the number of analytes detected. Some of these analytes include peptides, cells, enzymes and nucleic acids. ECL biosensors are selective, simple, sensitive and have low detection limits. Traditional methods use ruthenium complexes or luminol to generate ECL. Nanomaterials can be incorporated into ECL biosensors to improve efficiency, but also represent a new class of ECL emitters. This article reviews the application of ruthenium complex, luminol and nanomaterial-based ECL biosensors to making measurements in biological matrices over the past 4 years.

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ECL background

Electrogenerated chemiluminescence (ECL) [1–3], also termed electrochemiluminescence, is the production of photons from chemical reactions where the reactants are electrochemically generated. From a bioanalytical perspective, ECL combines the sensitivity of luminescence with the low cost of electrochemistry. ECL methods generally exhibit a wide linear range and low detection limits. The instrumentation required is inexpensive and straightforward to implement. In fact, commercial ECL-based systems have been developed that perform immunoassays and DNA probe assays [2–4]. However, research continues to find new ECL emitters, improve the bioanalytical methods and to apply ECL detection methods to new analytes and clinically relevant samples. This article reviews the application of ruthenium complex, luminol and nanomaterial-based ECL biosensors to making measurements in biological matrices over the past 4 years.

ECL reactions require a luminophore, also termed chemiluminescent reagent. Hundreds if not thousands of compounds have been demonstrated to participate in ECL reactions [3]. Many of these ECL reactions require aprotic and deoxygenated solution conditions. Therefore, only a few compounds and their derivatives are primarily utilized for aqueous-based ECL bioanalytical detection methods (Figure 1). These are luminol (5-amino-2,3-dihydrophthalazine-1,4-dione) and ruthenium(II) chelates with a general structure of RuL_3^{2+}, where L is the ligand. The structures of the two RuL_3^{2+} species discussed in this article are tris(2,2′-bipyridyl) ruthenium(II)dichloride ($Ru(bpy)_3^{2+}$) and dichlorotris(1,10-phenanthroline) ruthenium(II) ($Ru(phen)_3^{2+}$). In the 2000s, the ECL of nanomaterials was demonstrated. This review will focus on advances from the past 4 years on ECL biosensors applied to biological matrices using these three species and

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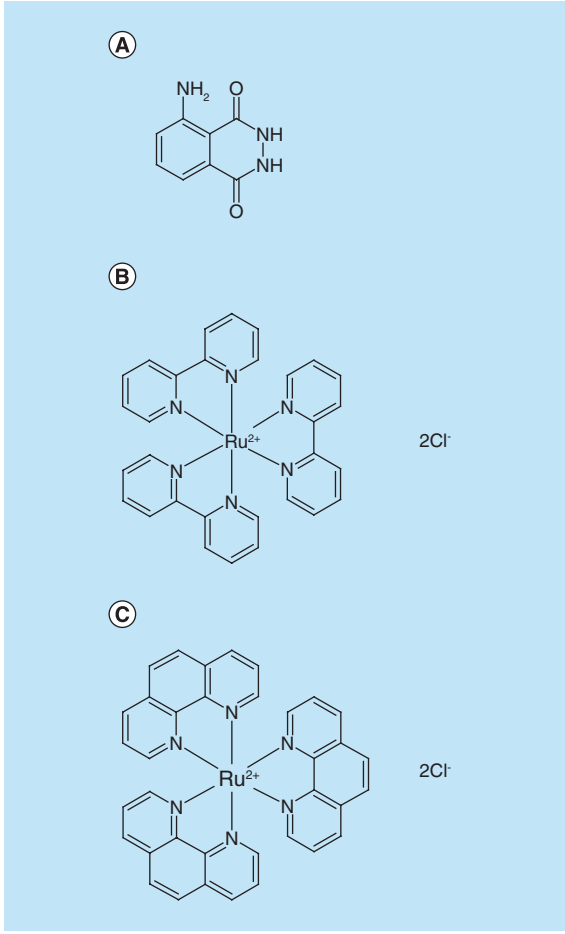

Figure 1. Chemical structures of common molecular luminophores discussed in this article. (A) luminol **(B)** tris (2,2′-bipyridyl)ruthenium(II)dichloride, and **(C)** dichlorotris (1,10-phenanthroline)rutheniu(II)

luminescent nanomaterials. The review will focus on advances from the past 4 years.

ECL reactions possess two degrees of selectivity through the requirements for one species to be electroactive and also participate in a chemiluminescent reaction. Additional selectivity can be achieved by producing biosensors. This additional selectivity is needed for making accurate measurements in complex matrices. A biosensor's selectivity is based on a specific biological interaction (e.g., antibody–antigen or enzyme–substrate) between the species being measured (target) and a recognition element. The biological response is converted to an analytical signal (e.g., current in electrochemical biosensors). For ECL biosensors, the signal is an increase or a decrease in light intensity.

The ECL signal is proportional to working electrode area, therefore considerable research has been done to increase the signal. Most of this work involves modification of the working electrode with species that enhance the signal, or modification of the working electrode to preconcentrate the chemiluminescent reagent. Additionally, work has also been performed to lower detection limits, improve selectivity and precision and to apply the methods directly to measurements in biological matrices.

Overview of RuL_3^{2+} coreactant ECL process

The ECL reactions of RuL_3^{2+} species have been studied since the 1970s [5–8]. In a deaerated aprotic solvent, RuL_3^{2+} can be both oxidized and reduced at an electrode (Equations 1–2). When both oxidized and reduced species are produced in solution, they can undergo an annihilation step to produce an electronically excited RuL_3^{2+} (Equation 3). The wavelength of maximum emission depends on the structure of the ligand. An excited $Ru(bpy)_3^{2+}$ solution emits orange light around 620 nm (Equation 4).

$$RuL_3^{2+} \rightarrow RuL_3^{3+} + e^- \quad \text{(Equation 1)}$$

$$RuL_3^{2+} + e^- \rightarrow RuL_3^{+} \quad \text{(Equation 2)}$$

$$RuL_3^{+} + RuL_3^{3+} \rightarrow {}^*RuL_3^{2+} + RuL_3^{2+} \quad \text{(Equation 3)}$$

$${}^*RuL_3^{2+} \rightarrow RuL_3^{2+} + h\nu \quad \text{(Equation 4)}$$

Bioanalytical methods based on RuL_3^{2+} ECL were not developed until the coreactant pathway was reported between $Ru(bpy)_3^{2+}$ and oxalate [9]. This type of ECL was generated in aqueous systems and was unaffected by the presence of oxygen. Probably the most relevant coreactant pathway is the 'oxidative–reductive' system between aliphatic amines such as tri-*n*-propylamine (TPrA) and RuL_3^{2+} [8,10–19]. In this mechanism, both RuL_3^{2+} and the coreactant are oxidized (Equations 5–6). The TPrA radical cation is not stable on the time frame of the experiment and quickly deprotonates, forming a free radical ($TPrA^{\bullet}$, Equation 7) This free radical behaves as a reducing agent and transfers an electron to RuL_3^{3+}, producing an excited state (Equation 8). Light is then produced as in Equation 4.

$$RuL_3^{2+} \rightarrow RuL_3^{3+} + e^- \quad \text{(Equation 5)}$$

$$TPrA \rightarrow TPrA^{+\bullet} + e^- \quad \text{(Equation 6)}$$

$$TPrA^{+\bullet} \rightarrow TPrA^{\bullet} + H^+ \quad \text{(Equation 7)}$$

$$TPrA^{\bullet} + RuL_3^{3+} \rightarrow {}^*RuL_3^{2+} + \text{products} \quad \text{(Equation 8)}$$

The equations above show the general mechanism between RuL_3^{2+} and TPrA leading to ECL. The specific steps depend upon both the potential applied

to the electrode and the relative concentration of the reagents [13,16]. Additionally, the coreactant and ruthenium complex are not always heterogeneously oxidized at the electrode [13,16,18–19]. When the electrode surface has been functionalized, the species are not always in direct contact with the electrode. In some cases, step 5 or 6 can occur homogenously. When the electrode is functionalized with conductive materials, these materials can facilitate and even catalyze electron transfer [18,19]. It was reported in the early 1990s that compounds with secondary and tertiary alkyl amine functional groups could serve as efficient coreactants and that the pH 7–8 range was optimal for generating a high signal [11]. At this point, the potential of this ECL reaction for bioanalysis was realized. The company Igen commercialized this technology for immunoassays and DNA probe assays [4]. In coreactant ECL, the signal is proportional to whichever species is the limiting reagent. Therefore, RuL_3^{2+} ECL can detect the coreactant as the analyte (e.g., biogenic amines in food) or the analyte can be labeled with the luminophore (e.g., immunoassays or DNA probe assays). RuL_3^{2+} can be introduced into the reaction chemistry via a few different methods. First, it can be present in solution and react with the analyte or a coreactant-labeled analyte. Second, a RuL_3^{2+} derivative can be used to 'tag' an analyte. Third, species such as $Ru(phen)_3^{2+}$ [7,8] can intercalate into the recognition element ('label-free' ECL). Finally, RuL_3^{2+} can be immobilized onto the electrode surface or attached to a substrate onto the electrode. Some ECL methods work via an enhancement of ECL signal (signal on) while others function via a quenching mechanism (signal off).

General biosensor formats & biosensor recognition chemistries

To fabricate a biosensor, the electrode must be modified in a way that makes it respond selectively to the analyte. A proper order of binding (or releasing) and ECL reactions must occur. A variety of formats have been developed, each with their own terminology and characteristics. Many of these formats seek to not only improve selectivity and sensitivity, but also develop miniaturized devices [20]. The following biosensor formats and chemistries have been used in the biosensors discussed in this review.

Direct probe

In the direct probe format, first an electrode is modified with a species that serves as both a capture and a signal probe, as shown in Figure 2 (top). This is a species that recognizes the analyte and has been labeled with the luminophore. When this electrode is placed in a solution of coreactant (e.g., TPrA) and the electrode properly biased, a strong ECL signal is observed. In the presence of the target (analyte), the target binds to the capture/signal probe and the ECL signal decreases. Generally, the decrease in ECL signal is proportional to the concentration of analyte.

Sandwich

In the sandwich format, the working electrode is modified with the capture probe. The electrode is placed in a solution of target that can bind to this capture probe. Next, signal probe (containing the luminophore) from solution binds to the target/capture probe. In the presence of coreactant such as TPrA, an ECL signal that increases with analyte concentration is observed. (Figure 2, bottom).

Label-free sensors

Label-free assays exploit the fact that $Ru(phen)_3^{2+}$ and some of its derivatives intercalate into dsDNA via electrostatic interactions. Methods based on intercalation do not require an extra chemical labeling step of the probe with luminophore. (Figure 3) Additionally, more than one chemiluminescent probe molecule intercalates into each dsDNA, resulting in a 'concentration' of $Ru(phen)_3^{2+}$ at the electrode surface. By contrast, in labeling methods only one $Ru(phen)_3^{2+}$ attaches to each probe molecule. When the electrode is placed in a solution containing target and a coreactant, an ECL signal is observed. The ECL signal increases in the presence of target if intercalation is promoted by its presence. Alternatively, as shown in Figure 3, the presence of target causes the release of luminophore from the dsDNA and a decrease in ECL intensity is observed.

Immunoassays

ECL immunoassay technology was developed by Igen (Origen Analyzer) in the 1990s [4]. The technology has since been purchased by Roche Diagnostics which markets the electrogenerated chemiluminescence immunoassay. The biotargets are not ECL-luminescent, so ECL labels of RuL_3^{2+} are needed. Generally, an antibody is immobilized onto a substrate, such as the electrode or the magnetic bead. The analyte is the target antigen and is captured by this antibody. A secondary antibody labeled with RuL_3^{2+} attaches to the antigen–antibody structure. In the presence of coreactant, ECL is observed. Sensitivity/amplification can be increased by incorporating the RuL_3^{2+} assembly into micro or nanostructures.

Aptasensors

In these sensors, aptamers function as the capture probe or sensing agent. They are generally attached to the working electrode and can sense anything

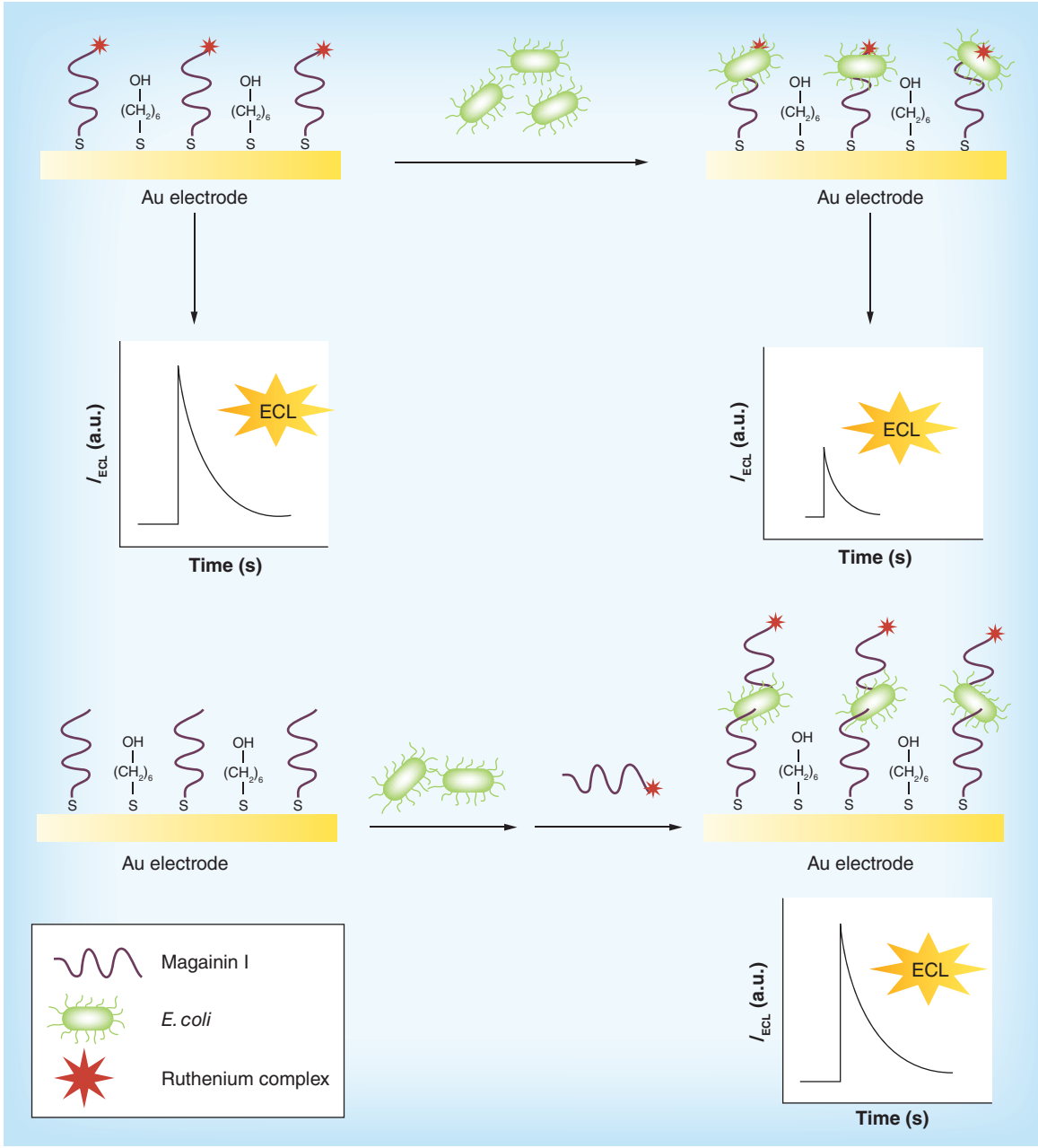

Figure 2. Diagrams of direct probe (top) and sandwich type (bottom) electrogenerated chemiluminescence biosensors for bacteria detection.
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from metal ions to small molecules, proteins, cells and bacteria. RuL_3^{2+} is attached to either the capture probe aptamer or to a secondary aptamer that serves as the ECL reporter (e.g., sandwich-type biosensors). An ECL signal is observed in the presence of coreactant and target. Amplification reactions can be used to increase sensitivity. Conversely, some aptamer-based ECL biosensors are 'signal-off' sensors in which ECL is quenched in the presence of the target. Label-free aptasensors have also been prepared. By exchanging the aptamer structure and thus the corresponding target, these methods can be modified to detect a wide variety of biomolecules. DNAzymes are catalytic nucleic acids that efficiently catalyze a variety of bioreactions. In addition to aptamers, DNA-zymes can function as the sensing agent. Addition of metal ion to these sensors decreases the ECL signal.

Magnetic beads

Magnetic beads are a means to physically manipulate the ECL detection reaction with a magnetic field. They typically are used to concentrate the luminophore in a

location close to the photon detector. They can be modified with many of the capture probes just discussed. After biorecognition has occurred, the beads can be concentrated onto an electrode and the ECL signal read.

Nanomaterials

Many groups have investigated the effects of incorporation of nanomaterials, such as nanoparticles (NPs) and quantum dots (QDs) into biosensors [23–28]. The unique mechanical, electrochemical and luminescent properties of nanomaterials have stimulated much research in this area during the past decade. Nanomaterials can improve the analytical performance of a biosensor, particularly the sensitivity and selectivity. First, they serve to increase the surface area of the working electrode. This increases the quantity of capture probes that can be immobilized onto the biosensor. Second, nanomaterials can produce electrocatalytic effects, increasing conductivity and facilitating electron transfer reactions. For example, anodic ECL of $Ru(bpy)_3^{2+}$ recently was reported with CdSe QDs acting as the coreactant for detection of DNA [29]. The strong ECL signal resulted from electrocatalytic effects on the oxidation of $Ru(bpy)_3^{2+}$ in the presence of coreactant. Third, nanomaterials can be functionalized with and transport conventional ECL luminophores such as luminol and ruthenium complexes. Finally, the nanomaterial can serve as the ECL luminophore. These properties will be discussed throughout the review and a final section is devoted to discussing electroluminescent nanomaterials.

Ruthenium complex-based ECL biosensor assays in biological matrices

Small molecules

Early reports of ECL-based detection methods were primarily for small molecules [5–6,9–15]. However, many of these methods lacked specificity and required a separation step prior to detection [14,15]. With the implementation of ECL-based biosensors, the selectivity required for making measurements in complex matrices has been achieved. Recent reports using ruthenium complexes for assays of small molecules in biological matrices are summarized in Table 1 and discussed here. The discussion will continue to include the detection of biological macromolecules in biological matrices, which are also included in Table 1.

Estradiol is a steroid and estrogen sex hormone used to treat menopause symptoms. A label-free competitive binding aptasensor was developed for estradiol (E2) in human serum and urine [30]. The biosensor chemistry was based on the competitive binding of E2 aptamer to E2 and cDNA. The aptasensor was constructed by modifying a gold electrode with an E2 aptamer. Both $Ru(bpy)_3^{2+}$ and a cDNA detection probe were present in solution. Under conditions of low concentrations of E2, the cDNA bound to the aptamer. $Ru(bpy)_3^{2+}$ intercalated into the aptamer and a strong ECL signal was observed. When the concentration of E2 increased, it competed with cDNA for binding sites on the aptamer, also displacing $Ru(bpy)_3^{2+}$. An inverse relationship was observed between ECL signal and E2 concentration. The biosensor exhibited a wide linear range from

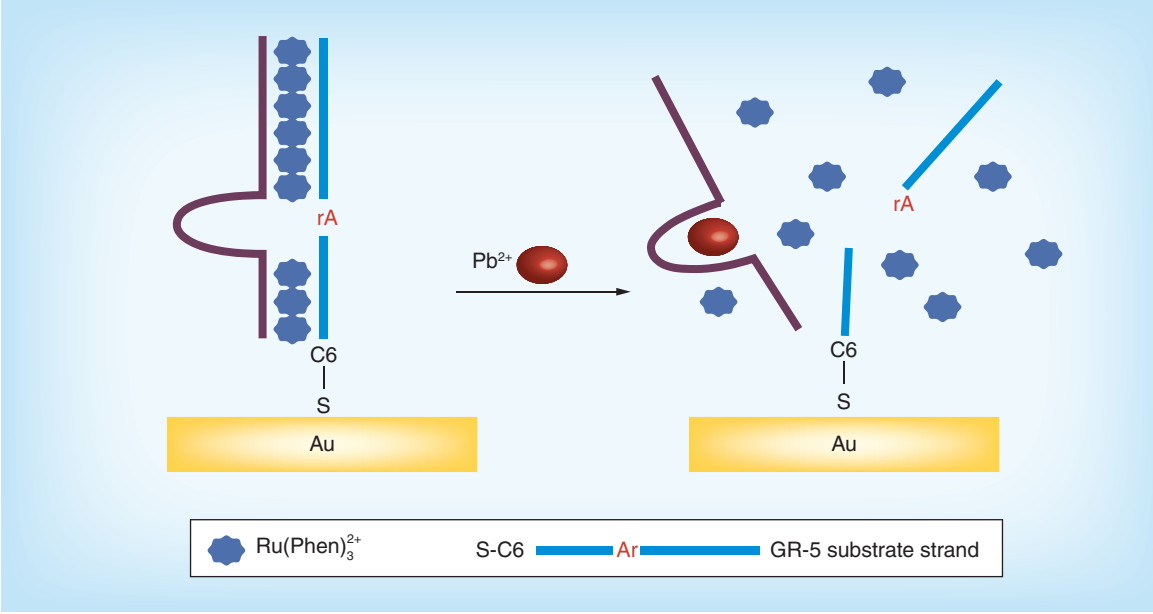

Figure 3. Diagram of a label-free electrogenerated chemiluminescence biosensor in which $Ru(phen)_3^{2+}$ intercalates into dsDNA.
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Table 1. Electrogenerated chemiluminescence biosensors based on ruthenium complex luminescence.

Analyte	Detection chemistry	Matrix	Detection limit/linear range	Ref.
DNA	Anodic ECL with CdSe coreactant	Human serum	0.19 fM 0.5 fM–5.0 pM	[29]
Estradiol	Label-free competitive binding assay using $Ru(bpy)_3^{2+}$ at Au electrode	Human serum and urine	1.1 pM 0.01–10 nM	[30]
Triodothryionine	Sandwich-type competitive immunoassay at GO-AgNP electrode	Serum	0.05 pg/ml 0.1 pg/ml–0.8 ng/ml	[31]
Lead	Intercalated-$Ru(phen)_3^{2+}$ ECL probe and the cofactor-dependent DNAzyme	Serum	0.9 pM 2–1000 pM	[22]
E. coli O157:H7	Ru1-ConA recognition probe on PIGE with SWNT; ECL quenching	Cells in buffer	127 cells/ml 5.0×10^2–5.0×10^5 cells/ml	[32]
E. coli O157:H7	Ru1-labeled AMP on Au electrode; both direct and sandwich format	Cells in buffer	2.3×10^2 CFU/ml (direct format) 1.2×10^2 CFU/ml (sandwich format)	[21]
Lysozyme	ECL aptasensor at Au electrode; $Ru(phen)_3^{2+}$ intercalation; low potential	Egg white	0.45 pM	[33]
Telomerase	Label-free $Ru(phen)_3^{2+}$ ECL probe	HeLa cell extract	2 HeLa cells 5–5000 HeLa cells	[34]
Carcinoembryonic antigen (CEA)	Addition of AuNP's formed Ru@SiO2-AuNP nanoarchitectures, giving ECL enhancement	Human serum	1.52×10^{-6} ng/ml	[35]
Prostate-specific antigen	$Ru(bpy)_3^{2+}$ ECL probe on AuNP/nafion/graphite pencil electrode	Serum	80 fg/ml 0.5–30 pg/ml	[36]
Ramos (tumor) cells	Bio-bar-code Fe_2O_3 nanoparticles	Whole blood	16 cells 0–500 cells	[37]
Cancer cells and surface N-glycan	$Ru(phen)_3^{2+}$ intercalation into ds-DNA; MoS_2 nanosheets; enhanced ECL dual detection	Human serum	150 cells/ml 10^2–10^6 cells	[38]
Pseudorabies virus antibody	Immunoassay; triply amplified at a glassy carbon-Au-graphene electrode	Swine serum	0.40 pg/ml 1 pg/ml–50 ng/ml	[39]
Brain natriuretic peptide	DNA aptamer-magnetic bead capture ECL sandwich assay	50% human serum	Low pg/ml	[40]

AMP: Antimicrobial peptide; ECL: Electrogenerated chemiluminescence; PIGE: Paraffin-impregnated graphite electrode; SWNT: Single-walled carbon nanotube.

0.01 to 10 nM with a detection limit of 1.1 x10-[12] M. Method recoveries of 89.8–100.0% and 90.0–103.5% were determined in human serum and urine, respectively. The method was shown to be both rapid and sensitive.

A biosensor for triodothryionine (T3) in serum with potential clinical use was reported [31]. T3 is the primary marker of thyroid function. This sensor employed a sandwich-type competitive immunoassay and utilized nanomaterials for analytical improvements. Graphene oxide (GO) nanosheets modified with silver NPs served as the working electrode and captured the target T3 via streptavidin binding. An ECL signal was observed when anti-T3 antibody-labeled $Ru(bpy)_3^{2+}$ attached to the bound T3. The ECL signal linearly increased with T3 concentration. In this study, the functionalized GO provided both high ECL probe loading and an increase in conductivity. Additionally, the anode was appropriately biased so that electrophoresis could be used to concentrate the ECL probe at the anode prior to signal collection. T3 was detected over a concentration range from 0.1 pg/ml to 0.8 ng/ml with a detection limit of 0.05 pg/ml. T3 was detected in serum with high specificity.

Lead is a toxic heavy metal and exposure during childhood has been linked to many brain disorders. Current lead detection methods such as atomic spectroscopy and anodic stripping voltammetry can be expensive, time consuming and may require extensive sample preparation. A label-free aptasensor was developed to measure trace amounts of lead in serum samples [22]. As shown in Figure 3, thiol-modified GR-5 DNAzyme (catalytic DNA) substrate self-assembled onto a gold electrode. cDNA hybridized with the DNAzyme (the recognition spot) and the

ECL probe $Ru(phen)_3^{2+}$ intercalated into the dsDNA. In the presence of TPrA, an ECL signal was generated as the $Ru(phen)_3^{2+}$ was oxidized at the electrode. In the presence of lead, the DNAzyme cleaved specific spots on the cDNA substrate, and broke up the dsDNA, releasing $Ru(phen)_3^{2+}$. The decrease in ECL intensity correlated with lead concentration. An ultralow detection limit for lead of 0.9 pM was determined. This method was simple and less costly than traditional lead determination methods such as anodic stripping voltammetry and atomic spectroscopic methods.

Bacteria

Fast and inexpensive methods are desirable for bacteria identification and disease detection. Bacteria detection using ECL biosensors was first reported 20 years ago and methods have continually improved [41–43]. Subsequently, work has progressed to improve the speed, sensitivity and selectivity of bacteria biosensors [21,32]. Sensors for *Escherichia coli* discriminated between Gram-positive and Gram-negative bacteria and did not require a separation and enrichment step. Using the protein lectin as the biorecognition element [32], a highly sensitive sensor with a fast response rate was developed by the direct ECL probe approach. A subsequent study [21] utilized RuL_3^{2+}-antimicrobial peptides as capture and signal probes (Figure 2). Both the direct and sandwich formats were investigated and found to have satisfactory performance. The selectivity of the device improved with the use of antimicrobial peptide, and both devices exhibited a high degree of specificity. These assays were applied to cells in buffer solutions but have not yet been applied to serum or other biological matrices.

Enzymes & enzyme activity

A novel biosensor was developed that required a much lower applied potential than other typical biosensors [33]. The authors noted that the higher oxidation potentials required by many ECL coreactant methods could damage many biomolecules. An aptasensor for the enzyme lysozyme was developed and applied to the direct analysis of lysozyme in egg white. A single-stranded antilysozyme aptamer was attached to a gold electrode. This strand formed a double strand with its complementary strand. The ECL probe $Ru(phen)_3^{2+}$ intercalated into the dsDNA. In the presence of lysozyme, the $Ru(phen)_3^{2+}$ was released and reacted with TPrA in solution. The authors found that the microenvironment inside the dsDNA allowed for the low potential condition. The environment both preconcentrated TPrA and facilitated its deprotonation and reaction. They found an intraday precision of 5% along with a high stability and regeneration of the aptasensor.

Telomerase is a basic ribonucleoprotein reverse transcriptase associated with the production of tumors in many types of cancer. Detection of telomerase is important for the early detection and diagnosis of many cancers, as it is a ubiquitous tumor marker. ECL methods offer a faster detection method than their PCR-based counterparts. A label-free ECL biosensor was developed that measured the activity of telomerase extracted from human cervical cancer HeLa cells [34]. A gold electrode was modified with thiolated telomerase substrate (TS) primer. Next, an extension reaction was performed with the extracted telomerase. The extended primer subsequently hybridized with cDNA, forming dsDNA fragments on the electrode. A $Ru(phen)_3^{2+}$ ECL probe intercalated into the dsDNA. Enhanced ECL was observed in the presence of HeLa cells. The response was linear with the number of cells over a range of 5–5000 cells. The detection limit of the method was two cells. Specificity was demonstrated against common interferents. These sensors were stable, simple and possess the potential to be faster and more convenient than sensors using an ECL label.

Cancer detection/diagnosis

Additional ECL-based biosensors have been reported for the application of cancer detection and diagnosis [35–38]. Some sensors are constructed to detect a biomarker, as was described for telomerase. Other sensors directly respond to the presence of cancer cells. A new approach was reported for the 'ultrasensitive' detection of carcinoembryonic antigen (CEA) cancer biomarker as a result of ECL enhancement. This technology has been termed surface-enhanced ECL and used $Ru(bpy)_3^{2+}$-loaded NPs [35]. CEA is a glycoprotein produced by tumor cells and is a well-known tumor marker. Current detection methods include ELISA and electrochemical immunoassays. In this work, a gold electrode was modified with aptamers for CEA. After incubation in CEA-containing solution, the electrode was immersed in aptamer-modified nanoparticles (SiONPs and AuNPs). In the presence of CEA and coreactant, the aptamer-modified NPs aggregated onto the electrode surface, producing ECL. In the presence of CEA, as much as a 30-fold ECL enhancement was observed. The biosensor was shown to be selective in the presence of other proteins. In human serum, a detection limit of 1.52×10^{-6} ng/ml was obtained. This surface-enhanced ECL method reported the lowest detection limit for CEA assays.

An ECL biosensor was developed to detect tumor cells in whole blood samples [37]. These biosensors took advantage of the fact that certain aptamers can spe-

cifically recognize tumor cells. These aptamers were conjugated to magnetic bio-bar-code Fe_3O_4 NPs. The nanocomposites acted as the amplification station to initiate rolling circle amplification (RCA) through a strand displacement reaction in the presence of a $Ru(bpy)_3^{2+}$-derivatized probe. The RCA products were trapped at a magnetic electrode to perform the magnetic particle-based ECL detection in the presence of coreactant. This method detected as low as 16 Ramos cells. Selectivity was demonstrated in mixed cell samples and the method was applied to whole blood samples.

It is important for sensors to not only differentiate cancer cells from normal cells but also concurrently detect cancer biomarkers. An ECL biosensor was reported that simultaneously detected both cancer cells and their surface N-glycan [38]. A glassy carbon electrode was modified with MoS_2 nanosheets. The nanosheets increased the surface area of the electrode and increased the electron transfer efficiency. A capture DNA strand was also attached to the electrode. A $Ru(phen)_3^{2+}$ ECL probe intercalated into the dsDNA, providing an ECL signal in the presence of TPrA and application of a positive electrode potential. In the presence of cancer cells, the $Ru(phen)_3^{2+}$ probe was displaced and the ECL signal decreased. Additionally, the cell surface N-glycan could be detected when a negative potential was applied to a gold electrode modified with graphite-C_3N_4. The ratio of the ECL intensity between the negative potential and positive potential was successfully used to evaluate N-glycan expression of MCF-7 cell lines. The method was applied to cells in human serum.

Other disease detection/diagnosis

A triple-amplification ECL biosensor was fabricated for the detection of pseudorabies antivirus (PrV) in swine serum [39]. When trace detection is desired, a methodology such as this one can lower detection limits. The three strategies employed a stretch–stowage–growth mechanism. First, a glassy carbon–Au–graphene-modified electrode was used to increase the surface area and electrochemical activity, while binding biological samples, such as PrV. Next, the stowage step included incorporating silica NPs which carried $Ru(bpy)_3^{2+}$ and increased the loading capacity. The next growth step included the addition of a biotin–streptavidin (B–SA) conjunction. This step increased the amount of immobilized secondary antibody, leading to signal amplification. The growth could be increased and the signal tuned by adding B–SA units to the B–SA–B structure. The sensor responded to monoclonal PrV antibody with high specificity and was linear over the concentration range of 1 pg/ml to 50 ng/ml. A detection limit of 0.40 pg/ml was determined. The method was applied to swine serum samples and performed comparably to ELISA methods.

Brain natriuretic peptide (BNP) is a cardiac biomarker of heart failure. Heart ventricles secrete BNP into serum at trace levels during heart failure. Therefore, high-sensitivity assays for this biomarker are needed for diagnosis. Preliminary work was reported for a DNA aptamer–magnetic bead capture ECL sandwich assay [40]. In this sandwich-type assay, capture–aptamer magnetic beads conjugated with BNP, which in turn hybridized with reporter $Ru(bpy)_3^{2+}$-aptamer, which provided an ECL signal in the presence of TPrA. This work screened over 50 candidate aptamers for their binding affinity to BNP. The highest affinity candidates were screened with the $Ru(bpy)_3^{2+}$-aptamer for ECL assay potential. The top performers were used as both capture and reporter aptamers. The assay was shown to be sensitive and linear for measuring BNP in 50% serum samples.

Overview of luminol ECL process

Luminol-based ECL [44,45] was first observed in the 1960s and is applicable to fabricating biosensors because the reaction detects hydrogen peroxide, a byproduct of many enzymatic reactions. The breakdown of H_2O_2 into reactive oxygen species (ROS) can further increase the ECL response. Some of the advantages of using luminol as an ECL luminophore include its low oxidation potential, low relative cost, nontoxic nature and high-luminescence quantum yield.

An established illustration is glucose detection. For example, in Equation 9, glucose oxidase (GOx) has been immobilized onto an electrode. It readily oxidizes glucose in solution, producing hydrogen peroxide. When luminol is oxidized in the presence of oxidant, it forms electronically excited aminophthalate ion that emits light around 420 nm (Equation 10). Many bioanalytical applications involve indirect quantitation of a substrate whose reaction enzymatically produces hydrogen peroxide [46].

$$\text{D-glucose} + O_2 \xrightarrow{\textit{glucose oxidase}} \text{gluconate} + H_2O_2 \quad \text{(Equation 9)}$$

$$H_2O_2 + \text{luminol} \xrightarrow{\textit{electro–oxidation}} \text{3-aminophthalate} + N_2 + h\nu \quad \text{(Equation 10)}$$

The mechanism for luminol ECL has been studied by various groups and depends on the oxidant species and voltage applied [44–49]. The reaction occurs most efficiently under alkaline (pH 8.0–8.5) conditions, when luminol has been deprotonated (pK_{a1} = 6.2). Most applications of luminol ECL focus on detection of the oxidant species, rather than labeling targets with luminol. At a properly biased electrode, and in the presence of both substrate and luminol, the amount

of ECL signal can be related to substrate concentration [45,46]. Other substrates that similarly produce H_2O_2 include substances such as choline, lactate and cholesterol [50–52]. In addition to these enzymatic-based biosensors, other biosensors have been produced utilizing biological reactions that produce hydrogen peroxide and other ROS. Table 2 summarizes the sensor chemistry along with analyte, linear range and detection limit for luminol-based ECL biosensors.

Luminol-based ECL biosensor assays in biological matrices

MicroRNA

miRNA are a class of short, single-stranded noncoding RNA sequences, usually 19–23 nucleotides [53,54]. Despite their small sequence, miRNA are thought to play a significant role in regulation of fundamental cellular processes such as transcriptional and translational regulation of gene expression [53–55]. MiRNA-155, in particular, has been established as a biomarker for certain types of cancer. Luminol-based ECL biosensors were used to detect the expression of miRNA-155 in cell lysates from various cell lines. These included human renal cubularepithelial cell line (HK-2), normal hepatocyte cell line (L02), human umbilical vein endothelial cell line and cervical cancer cell line (HeLa) (Figure 4) [53]. The technique first used dopamine (DA) to quench the luminol ECL signal. This step served to minimize the background signal and improved the sensitivity of the method. Next, a dual amplification procedure was implemented by Pb^{2+}-induced target recycling, leading to the formation of cleaved DNA fragments that acted as RCA primers to trigger the next amplification step. (Figure 4) The results indicated that miRNA has varying levels of expression in different cell lines. They were compared with those obtained from a commercial reverse transcription polymerase chain reaction kit. The study found that the two sets of data were in agreement, demonstrating that the method proposed by the study is an efficient way to determine the expression of microRNA for clinical analysis.

Another method for the detection of miRNA-155 used a bare glassy carbon electrode modified with AuNPs and a helper DNA [54]. This helper DNA was complementary to the hairpin DNA chains. When the target miRNA-155 was present, it formed extended dsDNA polymers with the hairpin hybridized chains. This displaced the hybridization chain from the immobilized helper DNA. The formation of these polymers promoted hemin intercalation. Hemin served to amplify the ECL of luminol by catalyzing the decomposition of H_2O_2 to ROS. The method was applied to human blood serum samples spiked with miRNA-155. The method had a linear range between 5 fM and 50 pM and a detection limit of 1.67 fM.

One limitation of current ECL biosensors is the possibility of false positive and false negative errors. These errors result from instrumental efficiency, environmental noise and the fact that ECL detection is based on intensity changes of a single emitter [55]. A newer method for detection of miRNA used ratiometric ECL biosensors. Utilizing the ratio of two signals instead one value, this approach minimized interference while increasing sensitivity. This study was the first to apply this technique to the detection of mi-RNA. This approach utilized the catalysis of luminol-modified platinum nanoparticles, which provided a large cross-sectional area for absorption. The study verified this technique by measuring the concentration of miRNA-21 in HeLa cell extracts and found it to be 2.1×10^{-3}, a value consistent with previous studies. A limitation of this method was its specificity. The ECL response was linear between 0.5 and 50 fM with a detection limit of 0.24 fM. This technique has potential application to immunoassays, DNA detection, cancer screening and monitoring environmental changes.

DNA detection

DNA detection is lucrative due to the many applications in clinical research. Sequence-specific DNA detection could help identify markers for genetic diseases and characterize viruses, bacteria and parasites. A new development in ECL-based DNA detection used hybridization chain reaction (HCR) to design a highly sensitive DNA biosensor [56]. The 5′ terminus of the tDNA hybridized with an immobilized captured probe, and the 3′ terminus hybridized with a concatomer while self-assembling into a long DNA-concatomer. This long concatomer was loaded with AuNPs which catalyzed a luminol-based ECL signal. With HIV-1 as a model analyte, this method was used to measure tDNA concentration. This biosensor exhibited a linear response from 0.02 to 1.0 pM with a detection limit of 5 fM.

Another objective in DNA analysis is the development of user-friendly methods and instruments for use by nonspecialists. Recently, a device with these characteristics was reported for detecting base pair mismatches (Figure 5) [57]. It utilized a DNA array for the visualization of SNPs. A bipolar electrode array [58,69] was used to generate ECL. Luminol-platinum nanoparticles were modified with various monobases and served as the ECL probes. DNA probe was attached to the anodic poles of the array. When the electrodes were exposed to the monobase-luminol-platinum nanoparticle, hybridization could occur. The ECL signal could be measured with a digital camera. A linear log–log

Table 2. Electrogenerated chemiluminescence biosensors based on luminol luminescence.

Analyte	Biosensor chemistry	Matrix	Detection limit/linear range	Ref.
microRNA-155	Pb^{2+}-induced DNAzyme-assisted target recycling and RCA	HeLa cells	0.3 fM 1.0 fM–100 pM	[53]
microRNA-155	HCR coupled with hemin intercalation as luminol the signal enhancer	Human blood serum	1.67 fM 5 fM–50 pM	[54]
microRNA-21	Luminol enhanced by gold particles	HeLa cell extracts	0.24 fM 0.5–50 fM	[55]
HIV-1 DNA	HCR coupled with AuNPs signal amplification	1% serum	5.0 fM 0.02–1.0 pM	[56]
DNA (nucleotide polymorphisms)	Bipolar electrode array with luminol-PtNP's modified with DNA monobases	Beta thalassemia blood samples	2 pM 2–600 pM	[57]
Platelet-derived growth factor	Sandwich-type aptasensor based on glucose-modified gold nanoparticles	Human serum and urine	17 fM 0.10 pM–0.50 nM	[58]
Thrombin	CdSe@ZnS QD resonance energy transfer	Human blood serum samples	1.4 fM 10 fM–100 pM	[59]
Thrombin	Ratiometric aptasensor: ECL quenching of QDs with luminol signal enhancement	Serum	2.2 fg/ml 0.5 pg/ml–100 ng/ml	[60]
Glucose	Electrocatalysis by MWCNT modified with CoNPs	Normal human serum sample	50 nM 0.5–600 μM	[61]
Glucose	Signal enhancement at electrode modified with Pt nanoflowers/ graphene oxide/glucose oxidase	Blood serum	2.8 μM 5–80 μM; 80–1000 μM	[62]
Glucose	Perovskite $LaTiO_3$–Ag 0.1 nanocomposite (catalytic oxidation of glucose)	Blood	2.50 nM 0.01 μM–0.10 mM	[63]
Acetylcholinesterase	Biosensor modified with Pd–Au nanowires	Human blood serum samples	0.0083 U/l 0.025 U/l–25 KU/l	[64]
Acetylcholinesterase	Graphene–Au–chitosan nanocomposite electrode with biofunctional biocomposite	Human serum	2.2 nM 6.7 nM–0.92 mM	[65]
Protein kinase	Signal amplification from AuNPs with Xanthine oxidase	10% fetal bovine serum	0.09 U/ml 0.1–10 U/ml	[66]
Amyloid B	Immunosensor with luminol-modified Ce–ZnO nanoflowers	Diluted serum	52 fg/ml 80 fg/ml–100 ng/ml	[67]
Cholesterol	Cathodic ECL at 3D-MoS_2polyaniline nanoflowers with Ag nanocubes loaded with cholesterol oxidase	Diluted human serum	1.1 nM 3.3 nM–0.45 mM	[68]

CoNP: Cobalt nanoparticle; ECL: Electrogenerated chemiluminescence; HCR: Hybridization chain reaction; MWCT: Multiwalled carbon nanotube; QD: Quantum dot; RCA: Rolling circle amplification.

relationship between the ECL image intensity and G–T mismatch target concentration was observed in the concentration range of 2–600 pM. This simple instrument was applied to beta thalassemia blood samples. It showed promise as an inexpensive and efficient method for detecting genetic disorders.

Platelet-derived growth factor

PDGF is a critical growth factor found in the platelets of humans [70,71]. It plays an important role in the initiation and progression of diabetic nephropathy (DN), and determining PDGF-BB levels can be useful in an early diagnosis of DN. PDGF also plays an

important role in the regulation of cell growth and division. PDGF-BB, specifically, is a good predictor of DN. For the detection of PDGF-BB, one group recently reported a sandwich-type luminol ECL aptasensor based on glucose modified AuNPs [70]. The first step of this technique used multilayered AuNPs and electrochemically reduced graphene to form a nanocomposite film on a glassy carbon electrode. This nanocomposite increased conductivity, resulting in promoted electron transfer and amplification of the ECL signal. The AuNPs were functionalized with GOx, which served as the signal probe. In the presence of glucose, GOx produced H_2O_2, and high sensitivity was achieved from the catalytic activity of the film. This technique was used to determine the PDGF-BB levels in human urine and serum samples from healthy subjects and subjects suffering from DN. The results indicated that subjects suffering from DN had higher levels of PDGF-BB than the healthy subjects. To determine the sensitivity of the technique, the study also measured PDGF-BB concentrations in human serum and urine samples spiked with PDGF-BB. The technique was highly sensitive with good selectivity. The linear range for detection of PDGF-BB was 0.10 pM to 0.50 nM with a detection limit of 17 fM.

Thrombin detection

Thrombin is an enzyme that is administered during surgery to control blood clotting. During surgery, it is important to monitor the thrombin levels in a patient's blood and adjust accordingly. Recently, ECL methods for thrombin using nanomaterials have been reported [59,60]. A novel strategy for the detection of thrombin utilized electrogenerated chemiluminescence resonance transfer between luminol and CdSe@Zn QDs in neutral conditions [59] in a label-free aptasensor. When the glassy carbon electrode was modified with CdSe@Zn QDs, anodic luminol ECL was observed in the absence of coreactant (e.g., H_2O_2). Increasing the applied potential and modifying the glassy carbon electrode resulted in a stronger anodic luminol ECL signal. The method was applied to blood samples spiked with various concentrations of thrombin. The results indicated a promising method that was able to detect thrombin with enhanced sensitivity, a wide linear range and good selectivity.

Glucose detection

Numerous methods for glucose detection using luminol-based ECL biosensors have been reported over the past few decades. New methods, particularly those using novel NP-based materials, are still in development [61–63]. In one method, multiwalled carbon nano-

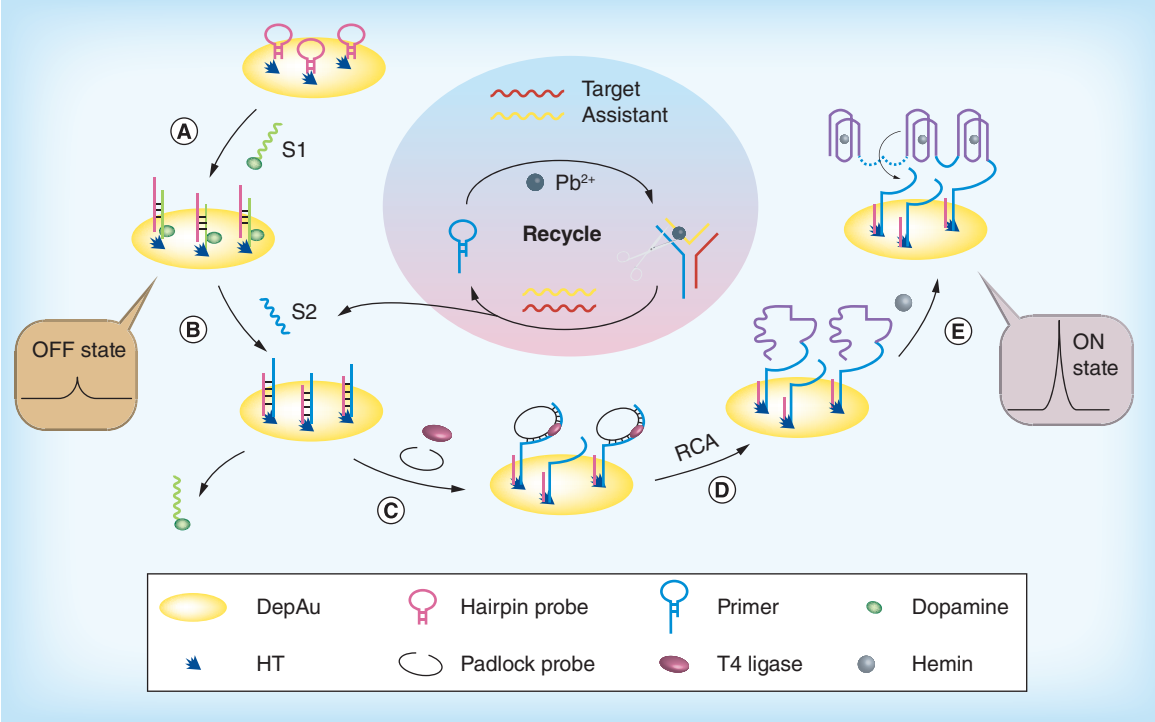

Figure 4. miRNA dual amplification electrogenerated chemiluminescence biosensor procedure. (A) Modification with DNA sequence 1 (S1) for off state, **(B)** Displacement of S1 by intermediate DNA sequence 2 (S2), **(C)** Incubation of T4 ligase and padlock probe, **(D)** rolling circle amplification **(RCA)** process, and **(E)** formation of hemin/G-quadruplex for on state.
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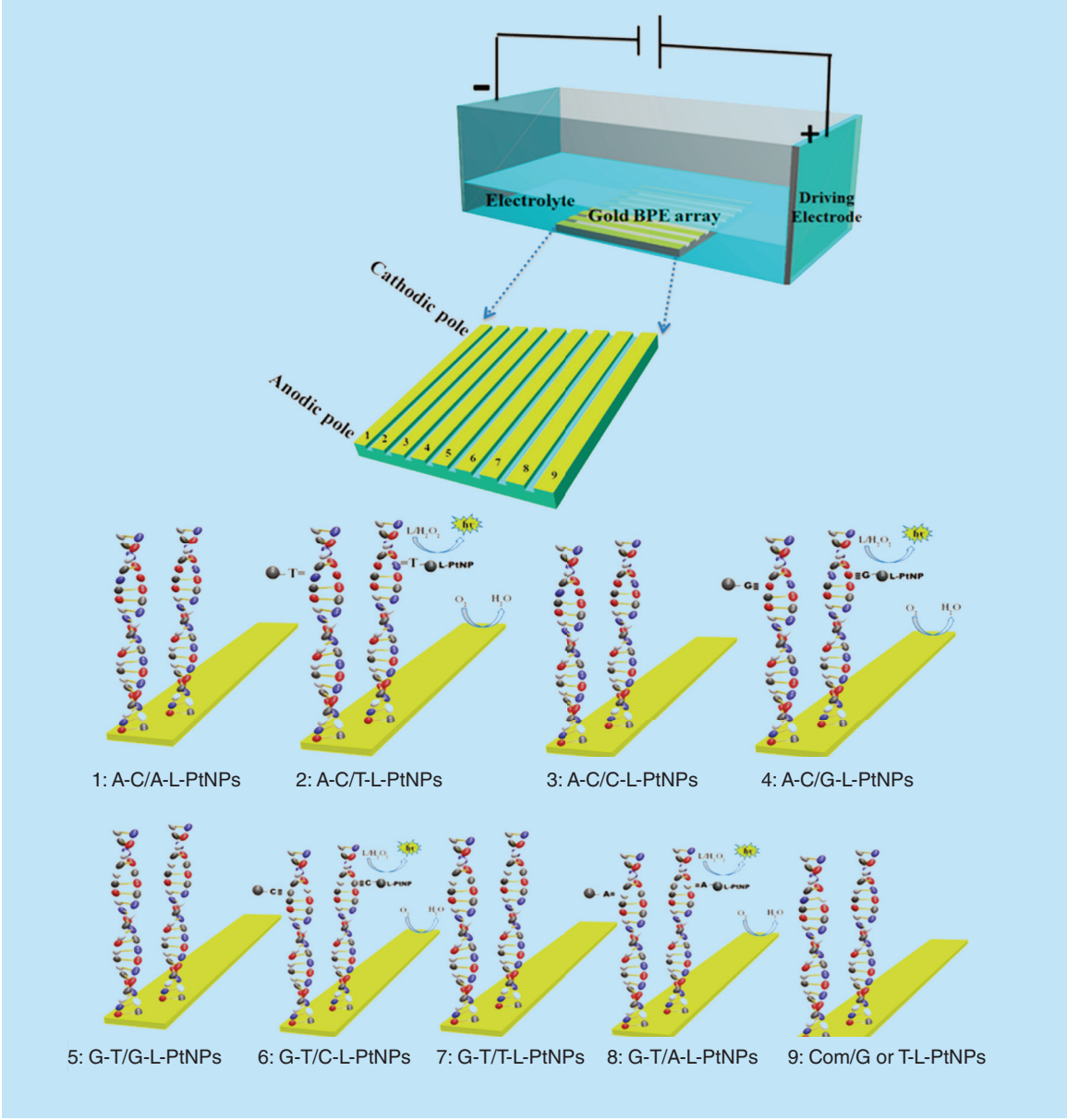

Figure 5. Biopolar electrode array with DNA targets.
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tubes were modified with cobalt nanoparticles and cast on a glassy carbon electrode in order to build a highly sensitive ECL sensor [61]. This electrode showed considerable electrocatalytic behavior for the luminol-H_2O_2 ECL reaction, especially at a neutral pH. This method was used to determine the glucose level in normal human serum. The linear range of the method was 0.5–600 µM with a detection limit of 50 nM. The analytical characteristics of other recent glucose detection methods are also reported in Table 2.

Acetylcholinesterase detection

Acetylcholinesterase (AChE) promotes the aggregation of amyloid-B peptides. This aggregation is often associated with the development of Alzheimer's disease. Recently two biosensors for AChE have been developed and applied to biological matrices [64,65]. An ECL biosensor modified with Pd–Au nanowires detected AChE in blood samples [64]. The Au–Pd nanowires increased the surface area for the immobilization of the bioenzyme AChE and choline oxidase, while sustaining signal amplification by providing catalytic activity of H_2O_2 oxidation. The suggested clinical applications for this method include protein analysis. A linear response was observed between 0.025 and 25 KU/l with a detection limit down to 0.0083 U/l. The results from the analysis of the blood samples support the practical use of this technique to monitor AChE activity in Alzheimer's patients.

Protein kinase detection

Understanding and measuring protein kinase activity has important ramifications in drug discovery. Protein kinases are involved in numerous biological processes including cell growth and communication. Recently, ECL biosensors have been developed to detect protein kinase [66,72]. One of these was able to detect PKA through multiple signal amplification probes [66]. The luminol/H_2O_2 ECL signal was enhanced with the use of AuNPs functionalized with xanthine oxidase. This modification catalyzed the reaction between hypoxanthine and dissolved oxygen to produce uric acid and H_2O_2. A clinical application of this biosensor is the detection of kinase in serum samples. PKA was spiked into fetal bovine serum samples and the activity of PKA was measured. The method was shown to be accurate and the ECL biosensor could be applied to complex biological samples. The detection limit of the biosensor was 0.09 U/ml, with a linear range between 0.1 and 10 U/ml.

Overview of nanomaterial ECL

In addition to enhancing the surface area and catalyzing electron-transfer processes during ECL, some nanomaterials can serve as the luminophore in ECL reactions [23–28]. The first report ECL from NPs was for silicon nanocrystal QDs in 2002 [73]. Subsequently, the ECL of many other types of nanomaterials, such as CdSe QDs and C_3N_4 nanosheets, has been demonstrated. The ECL processes are similar to those described earlier for molecular emitters. A NP valence band can donate electrons to an electrode (STEP 11) while the conduction band can accept electrons (STEP 12). ECL is produced via an annihilation route (STEPS 13–14).

$NP \rightarrow NP^{+\bullet} + e^-$ **(Step 11)**

$NP + e^- + NP^{-\bullet}$ **(Step 12)**

$NP^{+\bullet} + NP^{-\bullet} \rightarrow {}^*NP + NP$ **(Step 13)**

$^*NP \rightarrow NP + h\nu$ **(Step 14)**

As with ruthenium complexes, this route is not amenable to the aqueous oxygenated systems of biological matrices. Fortunately, NPs can also produce ECL via an oxidative–reductive coreactant route similar to steps 5–8 for ruthenium complexes. The coreactants used to generate NP ECL are the same species that are used for ruthenium complex and luminol ECL, including peroxydisulfate, triproplyamine, oxalate and hydrogen peroxide. Table 3 summarizes recent applications of ECL biosensors with luminescent nanomaterials to biological matrices. The next section highlights a few of these biosensors. Table 3 summarizes the sensor format along with analyte, linear range and detection limit for nanomaterial-based ECL biosensors.

Nanomaterial-based ECL biosensor assays in biological matrices

DNA & RNA

Methods for nucleic acid detection must be specific and sensitive, and a variety of nanomaterials have been employed not only as ECL emitters, but also as signal enhancers. QDs made from graphene (GQDs) have the advantages of being both biocompatible and non-toxic. They were used in an ECL sensor for miRNA that employed a dual amplification strategy, achieving detection limits of 0.83 fM [74]. Aminated 3,4,9,10-perylenetetracarboxylic acid (PTCA–NH_2) was used to immobilize the GQDs onto a glassy carbon electrode. Next, an Fe_3O_4–Au core–shell nanocomposite was used to immobilize DNA probes to the electrode. The Au catalyzed electron transfer, while the magnetic Fe_3O_4 allowed for separation. Helper DNA hybridized with the target miRNA and target recycling was used to enhance the ECL signal. The signal was additionally enhanced by the presenence of AgNPs. This dual amplification strategy was used to detect miRNA-155 in lysates from the following cells: Hela (cervical cancer cell line), L02 (normal hepatocyte cell line), HK-2 (human renal cubularepithelial cell line) and 22Rv1 (prostate carcinoma cell line). In another report, a novel DNA aptasensor was fabricated using an Au-paper electrode modified with graphene [75]. Luminescent silver NPs coated with calcium carbonate/carboxymethyl chitosan microspheres served as the ECL labels. Dual amplification resulted from AgNPs modified with cDNA. The device had fM detection limits and has promise for inexpensive portable gene diagnostics.

Proteins, enzymes & disease diagnostics

A variety of ECL assays for proteins, antibodies and enzymes have been designed and applied to biological matrices [76–81]. For example, CdSe QD ECL with amplification from a novel silver nanowire has been applied to immunoglobulin G (IgG) detection in serum samples [76]. IgG is an antibody that plays an important role in the immune system. This sensor used a 'signal on' sandwich immunoassay design. The key to the sensitivity of this sensor was the incorporation of the Ag nanowires into the single-walled carbon nanotube substrate. This structure was attached to an Au electrode and modified with a capture antibody. IgG in the sample bonded to the capture antibody, which was sandwiched by single-walled carbon nanotubes with emitting CdSE QDs. Additional methods have been

Table 3. Electrogenerated chemiluminescence biosensors based on nanomaterial luminophores.

Analyte	Biosensor chemistry	Matrix	Detection limit/ linear range	Ref.
miRNA-155	Graphene-QDs and Au@Fe_3O_4 with S_2O_8 coreactant and AgNP enhancement	Cell lysates	0.83 fM 2.5 fM–50 pM	[74]
DNA	Au-paper device with graphene and coated AgNP emitters/signal enhancement	Serum	8.5 aM 40 aM–50 pM	[75]
IgG	Sandwich immunoassay with CdSe SWNTs and Ag nanowires	Serum	1.0 pg/ml 1.0 pg/ml–0.5 ng/ml	[76]
Alkaline phosphatase	Inhibition of CdSe-NP/triethylamine coreactant ECL	Diluted serum	0.5 nM 0.5–6.4 nM	[77]
Alpha fetoprotein	AuNP + g-C_3N_4 nanosheet luminophores with $S_2O_8^{2-}$ coreactant	Serum	0.0005 ng/ml 0.001–5.0 ng/ml	[78]
CEA	CdS-graphene nanocomposites aptasensor with $S_2O_8^{2-}$ coreactant and AuNPs	Serum	0.01–10.0 ng/ml 3.8 pg/ml	[79]
CEA	Ratiometric CdSQDs and luminol/Pd nanocluster	Serum	1.0–100 pg/ml 0.62 pg/ml	[80]
CEA	Mn^{2+}-doped NaY_4:Yb/Er upconversion nanoparticles	Serum	5.2 pg/ml	[81]
Choline	Chox/polymer (PFO) dots with H_2O_2 coreactant	Serum	0.5 nM 1.25 nM–94.5 μM	[82]
Dopamine	DSP-CdTe QDs with O_2 coreactant	Cerebrospinal fluid	26 pM 50 pM–10 nM	[83]
Dopamine	Stabilizer-capped CdSe QDs with $S_2O_8^{2-}$ coreactant	Human urine Cerebrospinal fluid	3.0 nM 10 nM–3.0 μM	[84]
Glutathione (reduced and oxidized ratio)	QDs plus ZnAlDH nanosheets ERET sensor	Whole blood and serum	5 nM 0.025–0.5 μM	[85]
ATP[b]	Au-nanocluster-labeled hairpin DNA on QDs with DNAzyme cleavage; dual quenching	Serum	5.3 nM 10 nM–50 μM	[86]
ATP	Origami Au paper electrode with P-acid–Pt–Ag NPs and TPrA coreactant	Serum	0.1 pM 0.5 pM–7.0 nM	[87]

CEA: Carcinoembryonic antigen; Chox: Choline oxidase; ECL: Electrogenerated chemiluminescence; ERET: Electrochemiluminescence resonance energy transfer; QD: Quantum dot; SWNT: Single-walled carbon nanotube.

reported for alkaline phosphatase isoenzymes [77], alpha fetoprotein [78] and the tumor marker CEA [79–81].

Small molecules

Various small molecules have been detected using nanomaterial-ECL [82–87]. Choline is a metabolite involved in insulin regulation. An organic nanomaterial ECL biosensor was recently developed for choline detection in diluted serum samples [82]. Water-soluble poly(9,9-dioctylfluorenyl-2,7-diyl) (PFO) dots served as blue luminophores. They were electrostatically adsorbed onto a C_{60}-dendrimer material with choline oxidase, forming a nanocomposite on a glassy carbon electrode. The H_2O_2 produced in the presence of choline oxidase (similar to step 9) served as the coreactant for ECL production. The method was reported to have a lower detection limit (5.00×10^{-10} M) and wider linear range (1.25×10^{-9}-9.45×10^{-5}M) than other ECL and amperometric methods for choline.

Recently, methods have used CdTe and CdSe QD ECL to detect DA [83,84]. DA is a key neurotransmitter associated with various functions such as reward, movement and addiction. Unusually low levels of DA are found in people with Parkinson's disease. A recent study developed a highly sensitive method for DA detection based on CdTe QDs functionalized with 3,3′-dithiodipropionic acid di(N-hydroxysuccinimide ester) [83]. A glassy carbon electrode was modified with carbon nanotubes and a molecular recognition probe was immobilized onto the carbon nanotubes. In the presence of DA, the CdTeQDs were captured by the electrode and produced ECL with O_2 coreactant.

The method showed high specificity in cerebrospinal fluid, and had a detection limit of 3.0 nM and a linear range of 10 nM–3.0 μM. Other ECL biosensors utilizing nanomaterial-based emitters were developed for glutathione [85] and ATP [86,87].

Conclusion

Bioanalytical methods utilizing ECL-based biosensors continue to improve in terms of their analytical figures of merit and the range of analytes detected. Method detection limits have decreased to detect just a few cells in a single assay or to measure biomolecules at fM or pM concentrations. The incorporation of nanomaterials into the biosensors has helped increase the observed signal and efficiency of these devices. Nanomaterials also allow for customization of the sensor platform and have increased the variety of analytes detected by ECL methods, including cells, bacteria, nucleic acids and enzymes. Nanomaterials also offer a new class of ECL emitters. These methods are simple, inexpensive and sensitive. As methods have improved, they have been applied to biological matrices and demonstrate much potential for use in clinical diagnostics.

Future perspective

As healthcare trends move toward inexpensive yet quick and accurate tests, ECL-based biosensors have an opportunity to make an impact. An expansion of the assays to include more clinically relevant compounds and conditions is one significant direction. Currently, many of the analytical figures of merit for these methods are excellent. Improvements in selectivity will promote practical applications in biological matrices such as blood or urine. New developments in nanomaterials will allow sensors to be tailored with high specificity and electrochemical efficiency. Nanomaterial-based luminophores represent a new class of ECL emitters. Future work should focus on exploring the ECL performance of these new emitters. Additionally, work should also continue to investigate other clinically relevant analytes. A few multianalyte sensors have been developed, and work should continue in this direction. Additional areas for researchers to contribute to ECL biosensors include biological imaging, miniaturization (e.g., microfluidics) and high-throughput analyses.

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Executive summary

- Electrogenerated chemiluminescent (ECL)-based biosensors show much promise for clinical use and disease diagnosis.
- ECL-based biosensors are sensitive analytical devices with fast responses times.
- The operation of ECL biosensors is simple and costs are low.
- Nanoparticle technology has improved the efficiency and selectivity of ECL biosensors and are a newer class of ECL luminophores with much potential for developing novel biosensors.
- Biosensors fabricated with new biorecognition species have increased the number of analytes detected.
- Many ECL-based biosensor methods have been applied to biological matrices such as whole blood, serum and urine.

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