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REVIEW

Research on DNA Electrochemiluminescence Biosensing

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Abstract: Electrochemiluminescence (ECL) signal is generated directly or indirectly by the electrochemical reactions. Due to its characteristics of high sensitivity, specificity, easy realization of integration, real-time and in-situ detection, ECL detection provides powerful research tools and methods in life sciences at molecular level. Research on deoxyribonucleic acid (DNA) is an extremely important aspect in life science and shows the significance in the areas of forensic identification, epidemic prevention and environmental monitoring, especially for the early diagnosis and treatment of diseases. DNA-ECL biosensing is considered as the most promising technology for DNA analysis and has been a hot research topic in the fields of biology and medicine. This review summarizes the research on DNA-ECL biosensing technologies in China in the recent five years. Also, we look into its research prospects.

Key Words: Electrochemiluminescence, Deoxyribonucleic acid, Signal amplification

1 Introduction

Electrochemiluminescence (ECL) is a chemiluminescence process caused by electrochemical reactions directly or indirectly. In ECL detection, when a voltage or current is applied, the reactions occurred on the electrode either between the species or these species and other additional coreactants. Also, the redox reactions of luminophore produce some unstable species at intermediated states, resulting in the light-emitting. ECL method is the combination of the electrochemistry and chemiluminescence detection, and holds the features of both technologies, such as high sensitivity, good specificity, easy realization of integration, in-situ detection. In addition, the development of ECL is considerably improved by coupling with other technologies (for instance, high performance liquid chromatography, capillary electrophoresis, flow injection analysis[1–8]).

In the study of ECL, the research on ECL biosensing is a special concern of scientists and its application continues to be extended, especially in the clinical diagnosis. ECL method has been used for the related determination of tumor-markers and immunoassays[9]. DNA, the carrier of genetic information, plays an important role in the life sciences and clinical analysis. As early as 1990, Bard's group[10,11] focused on the interaction between DNA and ECL signaling molecules $Ru(phen)_3^{2+}$ and $Os(bpy)_3^{2+}$, and their application to the DNA sequence analysis. In recent years, DNA-ECL biosensing has attracted more attention. This review will present the development of this research field, mainly achieved by Chinese scientists.

2 DNA-ECL biosensing

The principle of DNA-ECL biosensing is based on the ECL signal variation caused by DNA hybridization. In 1991, ECL detection of $Ru(bpy)_3^{2+}$ was applied to immunoassays and specific DNA analysis by Blackburn[12]. Since then, DNA-ECL biosensing has been widely studied with $Ru(bpy)_3^{2+}$ and its derivatives as ECL labels. It's worth mentioning that Bard's group contributed a lot to the development of this research field (Fig.1)[13,14]. Besides, our group has made some achievement as well. The *N*-(4-aminobutyl)-*N*-ethylisoluminol (ABEI)-labeled DNA probe, proposed by our group, could identify accurately three-base mismatched sequences by ECL method and the detection limit of complementary sequences was 3.0×10^{-11} M[15]. The ferrocenecarboxylic acid-labeled probe DNA has

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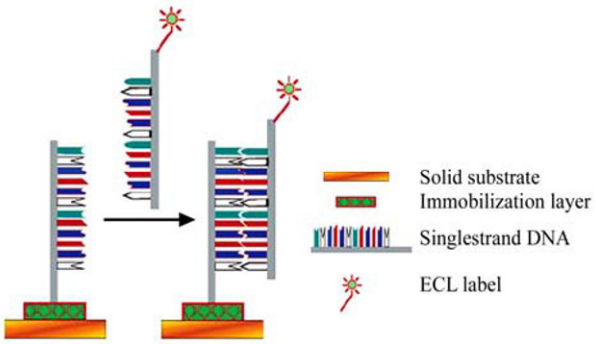

Fig.1 Schematic diagram of DNA hybridization detection using ECL (Reprinted with permission from American Chemical Society)[14]

also the satisfactory ability to recognize three-base mismatched and complementary sequences, based on ECL signal of luminol-H_2O_2 system, catalyzed by ferrocenecarboxylic acid[16].

Most DNA-ECL biosensing methods emploied linear DNA probes. In recent years, molecular beacon, as a sort of stem-loop ssDNA[17], has been increasingly utilized in the field of chemistry, biology and medicine[18–22]. Due to the complementary base pairs in its stem, the hybridization with target DNA needs to overcome the interactions between these base pairs. Accordingly, target DNA with high ratio of complementary bases is demanded, which can improve the detection specificity. In the presence of target DNA, the stem-loop structure of molecular beacons with the labels of ECL signaling molecules, could be converted into the linear double-strand DNA (dsDNA). As a result, the labeled end of probe moved away from the electrode surface, leading to a decrease of ECL signal (Fig.2)[23,24]. The responses of complementary and mismatched sequences are different significantly.

Wu *et al*[25] developed a reagentless DNA-ECL biosensing technology with the immobilization of ferrocene-labeled molecular beacon on thin oxide covered glassy carbon (C/C_xO_{1-x}) electrodes, which has intrinsic cathodic ECL characterization. The hybridization with complementary target increased the intensity of ECL signals and the single-base mismatched sequence can be effectively discriminated from the complementary one with the corresponding intensity ratio of about 0.7:1.

3 Applications of nanomaterials in DNA-ECL biosensing

Nanomaterials have attracted great interest as the novel functional materials in the 21st century meanwhile the application of nanomaterials to DNA-ECL biosensing becomes more and more popular. Nanomaterials are usually exploited to enhance the sensitivity in two ways: one is as the labels of DNA in the ECL methods; the other is as the supporter for the immobilization of ECL signaling molecules on electrode surface to increase their effective concentrations.

3.1 As labels of DNA

Nanomaterials as ECL signaling molecules, or the complex of nanomaterial and ECL signaling molecules can be directly or indirectly connected to the end of DNA via chemical bonding for the quantitative determination of target ssDNA.

Quantum dots (QDs) are one kind of new luminescent materials with unique photoelectric properties. Thus, as novel ECL luminophores, QDs have received more and more attention, and have been applied to the analysis of DNA and other biomolecules. Huang *et al* immobilized probe DNA on gold electrode. When biotin-labeled capture DNA hybridized with probe DNA to form dsDNA, avidin-labeled QDs as the ECL signaling molecules could be immobilized on dsDNA to produce ECL signals. This method is simple, convenient and timesaving, and showed a detection limit as low as 10 pM and high specificity[26]. Hu et al. reported a novel ECL technique on nanoporous gold leaf electrodes with mercaptopropionic acid-capped CdTe QDs, which attached the end of "sandwich" hybrids through amido bond. With $S_2O_8^{2-}$ as the coreactant, 5×10^{-15} M target DNA could be detected with good reproducibility[27]. In order to obtain higher sensitivity, Fe_3O_4@Au magnetic nanoparticles and QDs were labeled on the capture DNA and the signaling DNA respectively, by Hai Hong *et al*. The ultrasensitive detection of target sequences was realized through the construction of a sandwich structure on magnetic classy carbon electrodes[28]. QDs-dendrimer nanocomposites were synthesized by Divsar *et al* for ECL signal amplification. With molecular beacons as DNA probes, near single DNA molecules detection was achieved with a linear range of 7 orders of magnitude (Fig.3)[29].

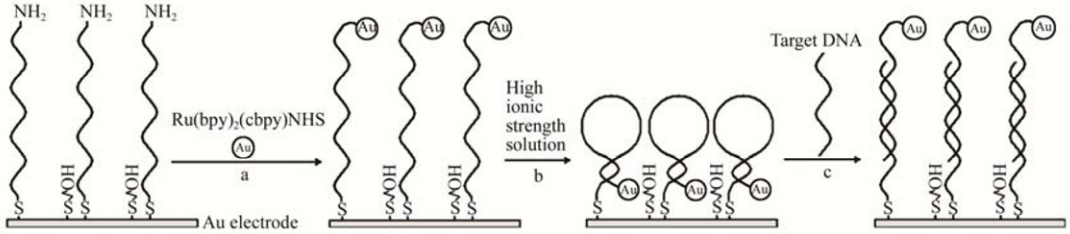

Fig.2 Schematic diagram of DNA-ECL biosensing for detection of DNA hybridization (Reprinted with permission from Springer)[24]

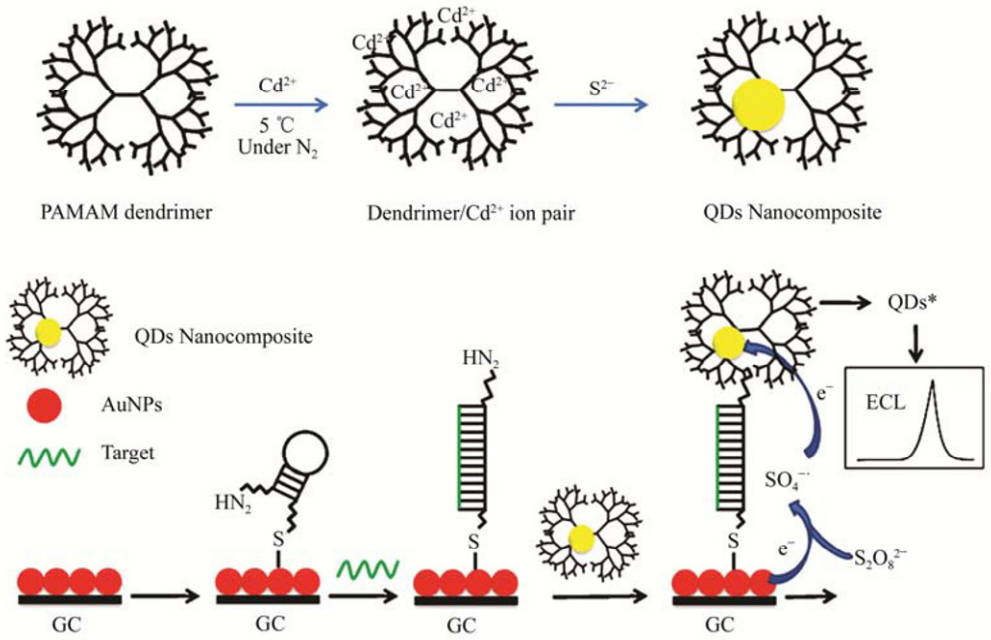

Fig.3 Schematic representation of ultrasensitive DNA detection on ECL biosensing platform labeled with QDs-dendrimer nanocomposite (Reprinted with permission from the Royal Society of Chemistry)[29]

Gold nanoparticles have also been applied to the labels of DNA-ECL biosensing to increase sensitivity. Wang et al. described gold nanoparticles as the carrier of $Ru(bpy)_2(dcbpy)NHS$-labeled probe DNA for the detection of target DNA, through which ECL signal was significantly amplified with a detection limit of 5.0×10^{-12} M[30]. Chai et al. chose luminol-functionalized gold nanoparticles as labels for ECL signal amplification, and highly sensitive detection of target DNA[31] and mycobacterium tuberculosis[32] were realized. In the research of Shan *et al*, gold nanoparticles were labeled on one end of molecular beacon and served both as ECL quencher and signal amplifier. The other end was immobilized on CdS:Mn nanocrystal film, one kind of ECL luminophore, whose signal was either quenched by proximal Au nanoparticles, or enhanced after the hybridization with target DNA. The detection limit could come to 50 aM[33]. With isothermal circular amplification reaction of polymerase and nicking endonuclease, the DNA detection limit decreased by an order of magnitude[34]. This approch succeeded in the detection of single nucleotide polymorphisms as well[35].

$Ru(bpy)_3^{2+}$-doped silica nanoparticles are another ideal labels in DNA-ECL biosensing, because silica nanoparticles can wrap a large number of $Ru(bpy)_3^{2+}$, resulting in effective signal amplication, and their surface can be easily functionalized with chemical groups, such as amino, mercapto, carbonyl group to connect biomolecules[36]. Our group utilized these nanoparticls as ECL signaling molecules to recognize specific DNA sequences. The amplified ECL signal led to a satisfactory detection limit of 1.0×10^{-13} M (Fig.4)[37]. $Ru(bpy)_3^{2+}$-doped silica nanoparticles were labeled on probe

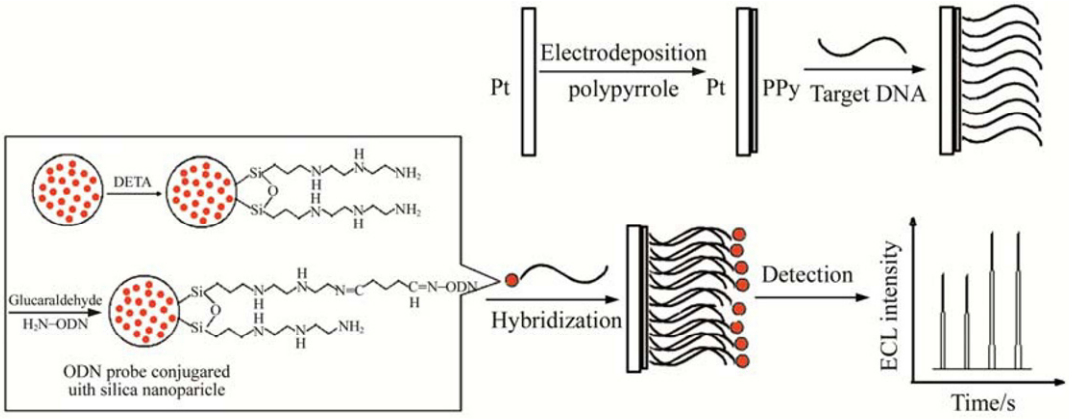

Fig.4 Schematic representation of (A) $Ru(bpy)_3^{2+}$-doped silica nanoparticles-labeled oligonucleotides probes and (B) the ECL detection of DNA hybridization based on the $Ru(bpy)_3^{2+}$-doped silica nanoparticles-labeled oligonucleotides probes[37]

DNA by Sun et al., which was modified on the electrode and presented the stem-loop configuration under high ionic strength. After the hybridization with target DNA, the stem-loop structure was straightened, leading to a weakened ECL signal, due to larger distance between the nanoparticles and the electrode. The detection limit was down to 5.0×10^{-14} M[38]. Afterward, this group proposed a duplex complex, formed by $Ru(bpy)_3^{2+}$-doped silica nanoparticles-labeled probe DNA, capture DNA and target DNA, which made the nanoparticles closer to the electrode surface and thus, ECL response appeared and 1.0×10^{-15} M target DNA was detectable[39].

Moreover, carbon nanotubes (CNTs) were also used in the labels of DNA-ECL biosensing. Li *et al*[40] used CNTs as the carrier of ruthenium compound and probe DNA for the determination of target DNA through the sandwich structure. Because CNTs carried a large amount of the ruthenium compounds, the signal was considerably amplified and the detection limit reached 9.0×10^{-15} M.

3.2 As the supporter for immobilization of ECL signaling molecules

The ECL signaling molecules prefer to be modified on the surface of electrodes, because it can decrease the reagent consumption and increase the effective concentration. As a consequence, the sensitivity and specificity are both improved. However, the leakage of ECL signaling molecules has a large impact on the sensitivity and stability of ECL methods. Therefore, a variety of new materials and immobilization methods are developed to solve this problem. Among them, nanomaterials show many excellent properties, such as good conductivity, large surface area, and special catalytic properties on the immobilization of ECL signaling molecules, which broaden the prospects for the application of DNA-ECL biosensing.

Our group reported a controllable solid-state ECL film based on $Ru(bpy)_3^{2+}$-gold nanoparticles composite, in which, gold nanoparticles served as the supporter of $Ru(bpy)_3^{2+}$. Ferrocene-labeled molecular beacon was fixed on this film *via* Au-S interaction (Fig.5). Due to the efficient quenching of ECL signal by the ferrocene, the presence of target DNA made the ferrocene away from ECL film, leading to a distinct change of ECL intensity. This highly sensitive and specific method was applied successfully to the detection of DNA[41,42], thrombin[43,44], T4 DNA ligase[45] and adenosine[46].

Wei *et al*[47] proposed the immobilization of $Ru(bpy)_3^{2+}$-doped silica nanoparticles on glassy carbon electrodes.DNA and other biomolecules were coated on the nanoparticles through the layer-by-layer assembly technique. With the increase of biomolecule coatings, the intensity of ECL signal decreased gradually because of the steric hindrance and limited diffusion of the coreactant. A label-free DNA-ECL biosensing was developed on the basis of CNTs/Nafion/$Ru(bpy)_3^{2+}$ composite film-modified electrodes by the same group. 3.04×10^{-8} M Salmon Testes-DNA and 3.93×10^{-10} M single-base mismatch of p53 gene sequence segment could be detected (Fig.6)[48]. Similarly, a multiwall carbon nanotubes (MWNTs)-poly(*p*-styrenesulfonate)- organically modified silicate composite film was applied to the immobilization of $Ru(bpy)_3^{2+}$ on glassy carbon electrode by Tao et al. Its determination limit of herring sperm dsDNA was 2.0×10^{-7} g mL^{-1}[49]. Our group coated the electrode surface with MWNTs-$Ru(bpy)_3^{2+}$ composite, on which gold nanoparticles-labeled DNA was immobilized *via* polypyrrole. Coupling the specific enzyme reaction of glucose dehydrogenase with ECL detection, a direct and accurate solid-state ECL biosensing platform was established for the detection of p53 tumor suppressor gene[50].

4 Applications of magnetic beads in DNA-ECL biosensing

Magnetic beads are great carriers for biomolecules and widely applied to the separation and purification of DNA, proteins, enzymes, cells, etc. In the study of Miao *et al*, $Ru(bpy)_3[B(C_6F_5)_4]_2$-loaded polystyrene beads worked as the label of target DNA, which hybridized with probe DNA fixed on the surface of magnetic beads. After the magnetic separation, CH_3CN was dissolved out of $Ru(bpy)_3[B(C_6F_5)_4]_2$-

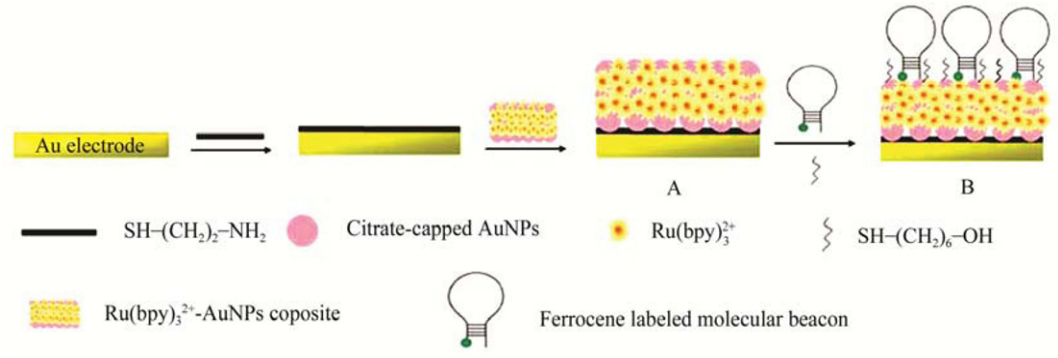

Fig.5 Schematic representation of (A) luminescent film of $Ru(bpy)_3^{2+}$-gold nanoparticles and (B) quenching monolayer on gold electrode[41]

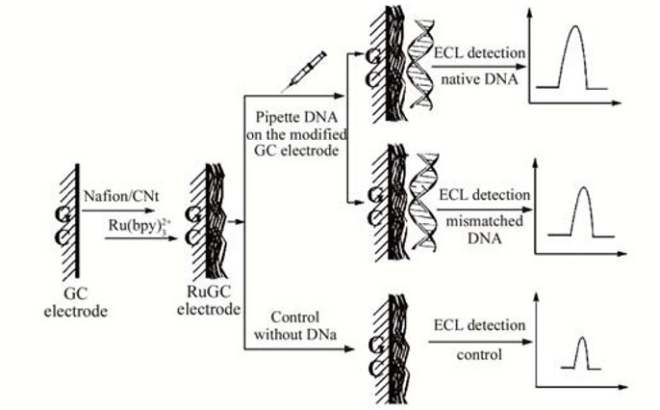

Fig.6 Schematic illustration of glassy carbon electrode modification and DNA-ECL detection procedures (Reprinted with permission from Elsevier)[48]

loaded polystyrene, the change of ECL signal of this process is used for the quantitative analysis of DNA with high sensitivity[51]. Shen et al. attached the streptavidin-coated magnetic nanobeads to the top of "sandwich" structure (capture DNA/target DNA/probe DNA), and these nanobeads were released with the addition of urea, followed by the conjugation with Ru(bpy)$_3^{2+}$-NHS. The composite was collected by magnet on gold electrode surface. The detection limit could reach 1.2 fM[52]. When the composite of magnetic submicrobeads and Ru(bpy)$_3^{2+}$ were wrapped with CNTs, 3 × 10^{-16} M target DNA was detected[53]. Zhou *et al* reported a novel approach for the analysis of point mutations in genomic DNA, in combination with allele-specific oligonucleotide ligation assay and magnetic beads-based ECL detection. The mutant target was complementary to two probes labeled with biotin and Ru(bpy)$_3^{2+}$, respectively. In its presence, these two probes could be connected by Taq DNA ligase. The connection would not be obtained when allele mismatches existed. The connection products were captured by the magnetic beads for ECL measurement[54]. With the assistance of rolling circle amplification technique, ultrasensitive detection of point mutations was achieved and 2 amol of mutated strands was detectable[55].

Polymerase Chain Reaction (PCR), as a technology of molecular biology for the amplification of specific DNA fragments, can be treated as special DNA replications *in vitro*. A great amount of work on ECL-PCR has been done by Xing's group[56–61]. Ru(bpy)$_3^{2+}$ and biotin were employed as the labels of PCR products, and they were attached to streptavidin-coated magnetic beads. Rapid determination of *Vibrio parahaemolyticus* in sea food was realized, through the analysis of the gyrase B gene by ECL method. Moreover, after PCR amplification, its product was digested by restriction enzyme, MvaI, enabling the achievement of K-ras and H-ras point mutations analysis. Recently, this group developed a nano-magnetic primer-based ECL-PCR method for genomic determination. Both PCR amplification and ECL detection were performed on the surface of nano-magnetic beads, which were utilized for the immobilization of primers for in situ PCR and enrichment of PCR products for in situ ECL detection. This method has the advantages such as excellent specificity and sensitivity[62].

5 Extended application of DNA-ECL biosensing

In recent years, the application of DNA-ECL biosensing has been extended to the detection of metal ions, small medical molecules, proteins and cells, through their interactions with DNA and aptamers special DNA sequences.

5.1 Aptamer-ECL biosensing

Aptamer, screened by SELEX (systematic evolution of ligands by exponential enrichment) technology, is a single-strand oligonuleotide segment, composed of 20−60 bases. It can combine with specific target molecules, including proteins, small medicine molecules, nucleic acids, and even whole cells, and form stable three-dimensional conformations such as G-quartet, hairpin, and bulge loop. In comparison with DNA-ECL biosensing, aptamer-ECL biosensing exploited aptamers as the recognition element, instead of ssDNA with no specific functions, resulting in the greatly extended application[63–65]. This technology has succeeded in the analysis of thrombin[66–77], lysozyme[78], small medical molecules[79–81], ATP[82–86], adenosine[87,88], cells[89–93] etc.

Sandwich structures, formed with gold nanoparticles-modified aptamer 1, thrombin, and Ru(bpy)$_3^{2+}$-labeled aptamer 2, were proposed for the determination of thrombin[72,77]. The signal amplification of gold nanoparticles enabled the thrombin detection with high sensitivity. Our group developed an aptamer-ECL biosensing method for the ultrasensitive determination of thrombin, showing the advantages such as fast response, satisfactory stability, sensitivity and specificity. In this method, CdS QDs-chitosan complex films were modified on the aligned carbon nanotubes electrode through the electrodeposition reaction of chitosan, and aptamers were connected to the film *via* glutaric dialdehyde. After the incubation with thrombin, an obviously decreased ECL signal was observed. The detection limit reached 5 × 10^{-14} M[74]. A novel dendrimers/CdSe-ZnS QD nanocluster was reported by Jie et al. for the analysis of cancer cells[89]. The aptamer, modified on the electrode, hybridized first with the dendrimers/CdSe-ZnS QD nanoclusters-labeled DNA probe. Then, in the presence of cells, the specific binding between cells and aptamers released the dendrimers, leading to the reduced ECL signals. Assisted by the cycle-amplifying technique on magnetic microbeads, the detection sensitivity was further improved.

5.2 DNA-ECL biosensing for determination of metal ions

Due to the specific interaction between DNA sequences and metal ions, a new technique for the detection of metal ions has been developed, recently. Through ruthenium compound-labeled DNA sequence containing thymine (T) and cytosine(C), the determination for Hg^{2+} and Ag^{+} could be successfully carried out[94]. T-Hg^{2+}-T and C-Ag^{+}-C complex was formed with unmatched T-T and C-C in dsDNA, respectively[95–98]. The interaction with Hg^{2+} and Ag^{+} made the DNA configuration changed, leading to the corresponding variation of ECL signals. Moreover, the detection of Pb^{2+} was also performed based on DNAzyme. Ruthenium compound-labeled dsDNA, formed with DNAzyme, was attached to gold electrode. With the addition of Pb^{2+}, dsDNA was dissociated into two pieces. Thus, the intensity of ECL signal changed, through which the ultrasensitive detection of Pb^{2+} was achieved[99,100]. To improve further the sensitivity and specificity of detection, Zhu *et al* labeled ssDNA with $Ru(bpy)_3^{2+}$-doped SiO_2 nanoparticles[101]; Li *et al*[102] immobilized $Ru(bpy)_3^{2+}$-labeled dsDNA for the recognition of Hg^{2+} on the surface of magnetic beads with the purpose of magnetic enrichment; Ma *et al*[103] took advantage of dendrimer as a carrier of $Ru(bpy)_3^{2+}$ to label the sequences, which could recognize specifically Hg^{2+}.

In the detection of metal ions, ECL signaling molecules can not only be labeled on DNA sequence, but also intercalate with the grooves of dsDNA. Yuan *et al* developed a label-free ECL biosensing method, in which, the intercalated $[Ru(phen)_3]^{2+}$ into dsDNA served as ECL signaling molecule for the determination of Hg^{2+}. In the presence of Hg^{2+}, the "supersandwich" was fabricated on the surface of gold electrode with the formation of T-Hg^{2+}-T complex. The intercalation of $[Ru(phen)_3]^{2+}$ into dsDNA enhanced the ECL response and a detection limit of 0.25 nM was obtained[104].

6 Prospects of DNA-ECL biosensing

DNA-ECL biosensing, as a simple and sensitive analytical technology, has been widely applied in the fields of clinical medicine, genetic engineering and the anti-cancer drug screening. Therefore, an increasing number of researchers focus on this study. The future development of DNA-ECL biosensing can be concluded as follows: (1) Preparation of novel and highly efficient ECL signaling molecules. For instance, new nanomaterials and multi ECL labels will be employed to produce more sensitive ECL signals, and maintain the bioactivity of analytes, simultaneously. (2) Exploitation of ECL signaling molecules immobilization techniques. The disadvantages of present ECL solid-phase electrodes include short lifetime, poor stability, and low sensitivity in solution. Consequently, it's necessary to exploit ECL signaling molecules immobilization techniques with high sensitivity and long lifetime. Meanwhile, direct recognition of biomolecules with electrochemical methods through homogenous DNA hybridization in solution without DNA immobilization is also in high demand. (3) Simultaneous determination of multi target DNA. The study shows great importance in the disease diagnosis, which requires the development of DNA-ECL biosensing arrays for the simultaneous determination of multi target DNA. (4) Coupling of ECL with various analytical methods. The use of coupling techniques will extend the application of each sub-technique and improve the performance. (5) Miniaturization of ECL analyzer. Due to the widespread application of ECL, the miniaturized ECL analyzer has important implications for the further development of life science, medicine and health science, and even for the economic development and national security of China.

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