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Review

Advances of Nanotechnology Applied to Biosensors

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Abstract

Up to date, application of nanomaterials and nanotechnology has made great advances. Many novel nanomaterials with unique properties are increasingly being exploited to apply for biosensors, improving the property of biosensor and making them higher selectivity and sensitivity, less response time and lower detective limitation. Here we review some of the main advances in this field over the past few years, explore the application prospects, and discuss the issues, approaches, and challenges, with the amin of promting to develop nanomaterials-based biosensor nanotechnology and improving their application in disease diagnosis and biosafety examination.

Keywords: biosensor; nanotechnology; gold nanoparticle; carbon nnaotubes; magnetic nanoparticles

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1. Introduction

Nanobiotechnology is defined as an interdisciplinary field of science that studies the application of fine-sized biological objects (of nanoscale, 1-100 nm) to design the devices and systems of the same size that utilize their unique effects for new purposes [1]. As the development of nanotechnology, the nanotechnology has been applied to biosensors such as nanoelectrodes and nanoelectrode arrays to improve their performance. Nanobiosensor, an integration of physical sciences, molecular engineering, biology, chemistry and biotechnology holds the possibility of detecting and manipulating atoms and molecules using nanodevices, which have the potential for a wide range of both industrial and domestic applications [2].

Herein, we review some of current main advances of nanotechnology applied to biosensors. Firstly, we give some examples of different nanomaterials such as gold nanoparticles, carbon nnaotubes(CNTs) and magnetic nanoparticles used in biosensors as electrode or membranes to enhance the biomolecule absorption or immobilization.Then we summary main types of biosensors such as electrochemical bosensors, immune biosensors, and giant magnetic resitance (GMR)- sensors. Lastly, we explore the application prospects, and discuss the issues, approaches, and challenges, with the amin of promting to develop nanomaterials-based biosensor nanotechnology and improving their application in disease diagnosis and biosafety examination.

2. The use of nanomaterials in biosensor

Nanomaterials such as gold nanoparticles, carbon nanotubes (CNTs), magnetic nanoparticles, etc. own great potential applications in biosensors owing to their unique properties [3-5]. In recent years, the application of these nanomaterials in biosensors have achieved great advances.

2.1 The use of gold nanoparticle in biosensor

The presence of gold nanoparticle in biosensor, provides a biocompatible microenvironment for biomolecules, greatly amplified the immobilized amount of biomolecules on the electrode surface, and improved the sensitivity of the biosensors [6-8]. And different structure of gold nanoparticle showed different property. The gold nanorods modified electrode layer showed better analytical response than gold nano particles [9].Bikash Kumar Jena et al [10].demonstrated the bioanalytical application of gold nanoparticles for the sensing of polyionic drugs, protamine and heparin by reversible aggregation/de-aggregation for the first time.

Non-crosslinking gold nanoparticle aggregation induced by the loss (or screen) of surface charges is applied for enzymatic activity sensing and potentially inhibitor screening [11]. Hui Wei et al [12]. described simple and sensitive aptamer-based colorimetric sensing of protein (alpha-thrombin in this work) using unmodified gold nanoparticle probes as shown in Fig. 1.

Glassy carbon electrode (GCE) was widely used in biosensor; modified GCE could adsorb gold nanoparticle to improve the activity of the biosensors [13]. Gold nanoparticle and methylene blue (MB) could be assembled layer-by-layer (LBL) into films on the GCE modified by thiourea for detection of human chorionic gonadotrophin (HCG) [14].Due to the high surface area of the nanoparticles for loading anti-HCG, the immunosensor would be a potential application on clinical determination of HCG level. And gold nanoparticle-modified electrode showed much wider pH adaptive range, much better electrochemical stability and larger response current to the reduction of H_2O_2 [15]. Ai Wu Shi et al [16]. demonstrated a simple and low-cost way for the fabrication of amperometric H_2O_2 biosensors based on in situ electropolymerization of PTHNWs–HRP–gold nanoparticle with the help of the AAO template.

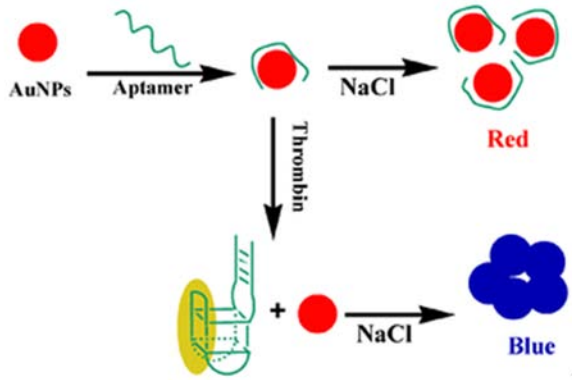

Fig. 1 AuNPs colorimetric strategy for thrombin detection[12].

Adoped with gold nanoparticle could improve the detection skill of bisosensor. For the first time, those GNPs/CNTs nanohybrids were used to construct an electrochemical immunosensor for highly sensitive immunoassay [17]. This developed label method was versatile, offers enhanced performances, and could be easily extended to other protein detection schemes as well as in DNA analysis. A nano-composite ZrO_2/Au film was prepared through a combination of sol–gel procedure and electroless plating that can be carried out in a general chemistry lab with no need for special facilities and reagents [18]. The specific strong adsorption of OPs on the homemade ZrO_2/Au film electrode surface provides an effective quantitative method for OP pesticides analysis.

2.2 The use of CNT in biosensor

Electrostatic interactions between a cationic surfactant/polymer and CNTs were utilized for the fabrication of biosensors [19]. Jinxiang Zeng et al [20]. described a way for fabrication of Prussian Blue nanoparticles/carbon nanotubes/poly (1,2-diaminobenzene) based glucose biosensor. The selectivity was greatly improved due to lower operation potential afford by the catalytic ability of PB/MWNTs nanocomposite toward reduction of hydrogen peroxide and the excellent anti-interference ability of PDAB film. An organic plasma polymer coating method [21]. provided a well-defined nanocomposite of enzyme, CNTs, and PPFs, the resulting device showed that the oxidizing current response due to enzymatic reaction was 4–16-fold larger than that with only CNT or PPF. Compared with the biosensor without carbon nanotube modification, the CNTs improved the sensitivity of the biosensor up to five times [22]. Hitoshi Muguruma et al [23]. reported an amperometric biosensor that is based on a nanocomposite of CNT, PPF, and GOx as an enzyme model. A mixture of the GOx and a CNT film is sandwiched with 10-nm-thick acetonitrile PPFs.

The bionanocomposite layer of multiwalled carbon nanotubes (MWNT) in chitosan (CHIT) can be used in the detection of DNA [24]. The biocomponent represented by double-stranded herring sperm DNA was immobilized on this composite using layer-by-layer coverage to form a robust film [25]. SsDNA probes could be immobilized on the surface of GCE modified with MWNTs/ZnO/CHIT composite film. The sensor can effectively discriminate different DNA sequences related to PAT gene in the transgenic corn, with a detection limit of 2.8 mol L^{-1} of target sequence.

Carbon nanofibers are found to be an effective strategy for building a biosensor platform [26]. Hui Ping Bai et al [27]. found that the synergistic effects of MWNTs and ZnO improved the performance of the biosensor. They reported an amperometric biosensor for hydrogen peroxide, which was developed based on adsorption of horseradish peroxidase at the GCE modified with ZnO nanoflowers produced by electrodeposition onto MWNTs film. Shu Ping Zhang et al [28]. described a controllable layer-by-layer self-assembly modification technique of GCE with MWNTs [29,30]. and introduce a controllable direct immobilization of acetylcholinesterase (AChE) on the modified electrode. By the activity decreasing of immobilized AChE caused by pesticides, the composition of pesticide can be determined.

2.3 Other materials used in biosensor

Aside from gold nanoparticle and CNTs, there are still various nanomaterials that could be used in biosensor to improve the property due to their nanosize. By using a simple in situ chemical reduction method Platinum nanoparticles (PtNPs) could be attached to glass slide surface [31]. Nano-CuO is used to enhance the electron-conductor instead of many other nano-materials and show good catalytic ability to the reduction of H_2O_2 [32]. Jiongjia Cheng et al [33]. investigated the promotion effect

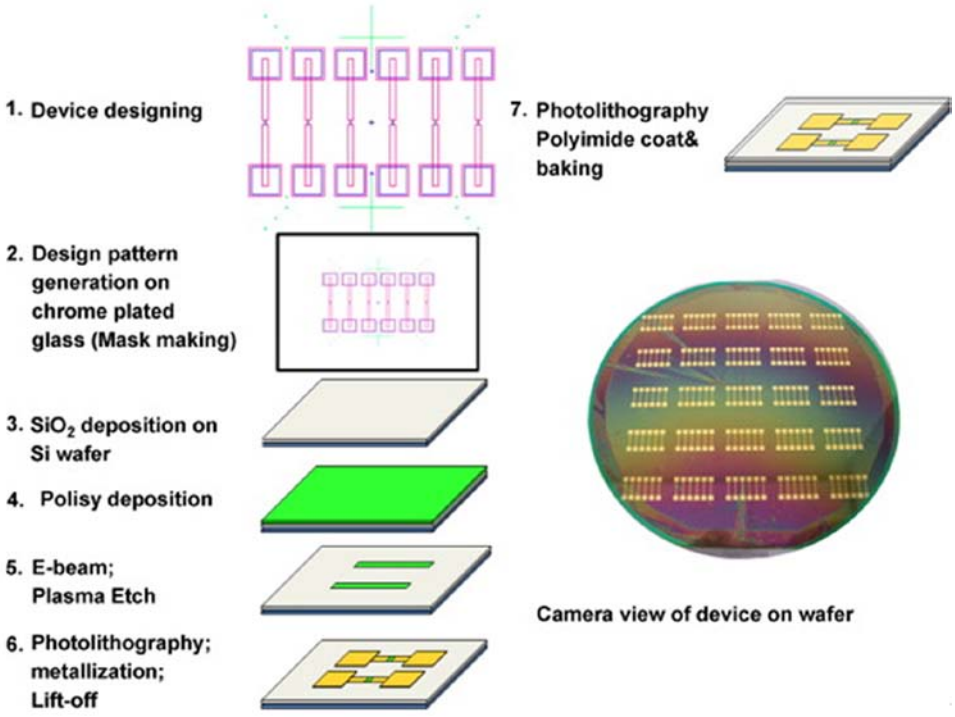

Fig. 2 Schematic diagram of process flow involved in nano-FET fabrication[46]

of nano-TiO_2 on the direct electron transfer between lactate dehydrogenase and the silica sol–gel modified gold electrode, and this electrode can be used as a biosensor for the determination of lactic acid. With liposome-based nanobiosensors, monitoring of the organophosphorus pesticides dichlorvos and paraoxon at very low levels has been achieved [34]. The nano-sized liposomes provide a suitable environment for the effective stabilization of AChE and they can be utilized as fluorescent biosensors.

Nanosheet-based ZnO microsphere with porous nanostructures was synthesized by a facile chemical bath deposition method followed by thermal treatment, which was explored for the construction of electrochemical biosensors [35]. It has been found that the waxberry-like ZnO balls acted as excellent materials for immobilization of HRP and rapid electron transfer agent for the fabrication of efficient biosensors due to their high electron conductivity, large surface area, increased oxygen vacancies, and good biocompatibility [36].

A 2-dimensional simulation tool was designed to investigate the threshold voltage behaviour for a silicon nanowire constructed in a top down approach on silicon on insulator (SOI) material [37]. The threshold voltage increases with decreasing thickness of the silicon nanowire, which could results in a higher sensitivity for surface charges.Surface functionalized Silicon nano-channels with the enzyme urease could detect and quantify urea concentration [38]. Nanofabricated biosensor from a SOI wafer with a top down lithography approach. The differential conductance of silicon nano-channels can be tuned for optimum performance using the source drain bias voltage, and is sensitive to urea at low concentration. In another work, SOI substrate is used to fabricate the planar type patch clamp ion-channel biosensor, which is suitable for the high throughput screening [39]. The channel current showing the desensitization unique to TRPV1 is measured successfully.

A novel disposable AFP immunosensor is proposed based on HRP/{nano-CdS/Thi+}2/PCR films modified GCE, The {nano-CdS/Thi+}2 bilayer films formed have high electroactivity and stability [40]. Nano-CdS is used to construct the {nano-CdS/Thi+} 2 bilayer films to result in larger electrode surface area to increase the immobilization of antibodies and improve sensitivity of the immunosensor.

2.4 Different material structures used in biosensor

Nanotechnology provides unique nanostructures such as metallic, semiconductor or magnetic nanoparticles, nanowires or nanotubes, exhibiting unique, size-controlled, electronic, optical and catalytic properties [41].

Devices based on nanowires have emerged as one of the most powerful and general platforms for ultrasensitive, direct electrical detection of biological and chemical species and for building functional interfaces to biological systems [42]. Fengyan ZHANG et al [43]. demonstrated the feasibility of using a CMOS compatible process in fabricating a submicron IrO_2 nanowire array biosensor platform. Biofets (biologically active field-effect transistors) are biosensors with a semiconductor transducer. Field-effect biosensors based on planar

structures and nanowires have been fabricated in recent experiments [44]. Masuhiro Abe et al [45]. showed that an NTFET biosensor can be calibrated using the Langmuir adsorption equation. Living molecules can be quantitatively detected by using any NTFET biosensor without precise control of the CNT growth.Wusi C. Maki et al [46]. reported the first nanowire field effect transistor based biosensor technology which achieves simple and ultra-sensitive electronic DNA methylation detection and avoids complicated bisulfite treatment and PCR amplification, the schematic diagram was showed in Fig. 2. Soon Joon Yoon et al [47]. investigated the target dependence of the sensitivity in a localized surface plasmon resonance biosensor and compare it with that of a conventional thin-film-based plasmon resonance structure. Sensitivity enhancement by LSPR using periodic nanowires has an SEF in the range from zero to over ten for various target interactions in water. And glucose oxidase could be deposited onto the nanowires using self-assembling monolayer as an anchor layer for the enzyme molecules [48].

Poly(ethylene-co-glycidyl methacrylate) (PE-co-GMA) nanofibers with abundant active epoxy groups on surfaces were fabricated through a novel manufacturing process [49].The results demonstrated that PE-co-GMA nanofibers prepared could be a promising candidate as solid support materials for potential biosensor applications. Kh. Ghanbari et al [50]. reported the use of NPV for the synthesis of PPy nanofibers on a platinum electrode. As a new electrochemical biosensor, this electrode is applied to study the interaction of SPD with DNA/PPy nanofiber films.

CdS hollow nanospheres (HS-CdS) are firstly used to study the direct electrochemical behavior of Hb and the construction of nitrite biosensor [51].The HS-CdS nanostructure provides a microenvironment around the protein to retain the enzymatic bioactivity.

With the use of the boundary element method, a general, practical map of the resonances for use in locating the desired response for gold nanoantennas was provided by calculating the nanorod near-field and far-field response to show how the nanorod shape and dimensions determine its optical response [52].A hetero-structured silicon/gold nanorod array fabricated by the glancing angle deposition method is functionalized with anti-Salmonella antibodies and organic dye molecules [53]. This bio-functional hetero-nanorod detection method has great potential in the food safety industry as well as in biomedical diagnostics.

Nanopore detector can be classified by using machine learning techniques such as Hidden Markov Models (HMMs) and Support Vector Machines (SVMs) [54]. These methods provide biologists and chemists a way to get better understanding of the kinetic properties of molecules of interest. By using a nanopore detector, two bifunctional aptamers could be examined [55]. The nanopore detector is biologically based and uses a protein,

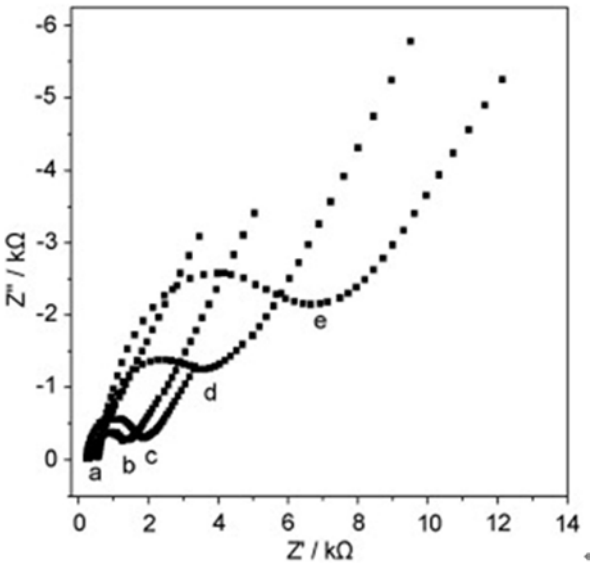

Fig. 3 Electrochemical impedance spectra of **(a)** bare Au; **(b)** Cys SAMs/Au; **(c)** CdTe–Cys SAMs/Au; **(d)** AChE–CdTe–Cys SAMs/Au and **(e)** AChE–Cys SAMs/Au recorded at potential of 0.20 V in pH 7.0 PBS containing 5 mM $K_3Fe(CN)_6$ and$K_4Fe(CN)_6$[58]

the α-hemolysin (α-HL) toxin produced by the bacterium Staphylococcus aureus, to create a pore through a phospholipid bilayer by self-assembly. Nanoscale tubes provide a higher exposed surface area compared to the flat surface electrodes. A novel approach is to use a nano-porous electrode to fabricate a nanotube array of PPy and thereby enhancing enzyme entrapment [56]. This results a high capacity of enzyme adsorption and enables fast transport of the gaseous by-product.

One of the fastest moving and most exciting interfaces of nanotechnology is the use of quantum dots (QDs) in biology. The unique optical properties of QDs make them appealing as in vivo and in vitro fluorophores in a variety of biological investigations, in which traditional fluorescent labels based on organic molecules fall short of providing long-term stability and simultaneous detection of multiple signals [57]. The nanoparticles of CdTe QDs led to an increased effective surface area for immobilization of enzyme and their electrocatalytic activity promoted electron transfer reactions and catalyzed the electro-oxidation of thiocholine, thus amplifying the detection sensitivity as show in Fig. 3 [58]. Zhengtao Deng et al [59]. reported green and orange CdTe QDs as pH-sensitive fluorescent probes could monitor the proton (H+) flux driven by ATP synthesis for dual simultaneous and independent detection of viruses on the basis of antibody−antigen reactions.

Sungkyu Seo et al [60]. constructed a biochip sensor system, consisting of two Ti contact pads and a 150 nm wide Ti nanowell device on $LiNbO_3$ substrate, show in Fig. 4. When the bacteria were resistant to the phages (uninfected bacteria), small voltage fluctuations were observed in the nanowell displaying a power spectral density (PSD) of 1/f shape.

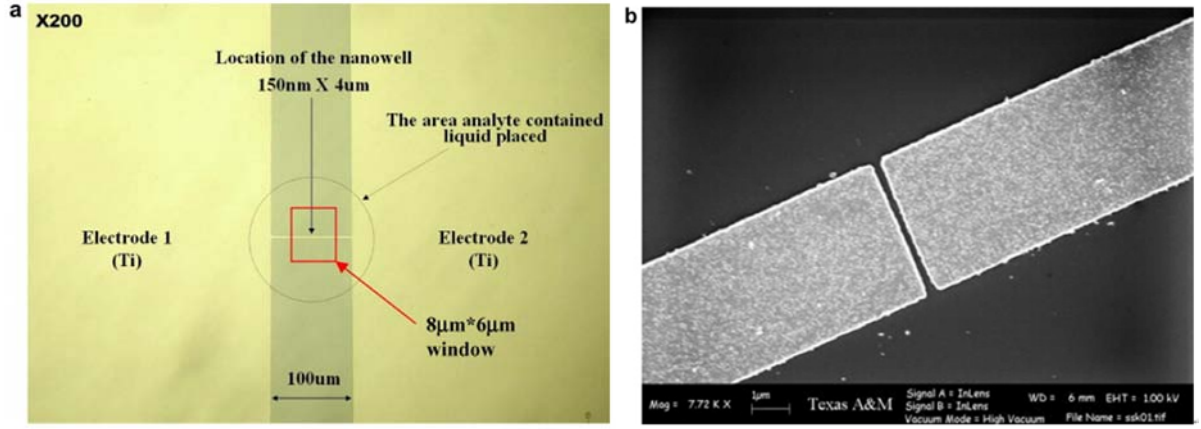

Fig. 4 (a) Optical microscope pictures of the fabricated nanowell device and contact pads (200×); **(b)** SEM picture of the nanowell (7720×). To improve image contrast, this nanowell was patterned on silicon substrate. The nanowell used in detecting bacteria/phage was patterned on LiNbO3 substrate [60].

3. Produce methods of nanobiosesors

There are different methods to product nanobiosensors for various uses. Here we list some major methods such as magnetism, electrochemical and immune method.

3.1 Magnetism method

The nanoscaling laws of magnetic nanoparticles, including metals, metal ferrites, and metal alloys ,are important not only for understanding the behavior of existing materials but also for developing novel nanomaterials with superior properties [61]. A microsphere (MDMS) with uniform shape and narrow diameter distribution has been prepared from magnetic iron nanoparticles and dextran [62].The successfully immobilized of Horseradish peroxidase on the surface of an MDMS-modified GCE lead to a fast response to H2O2 of less than 10 s and an excellent linear relationship .A magnetic chitosan microsphere (MCMS) was prepared using carbon-coated iron magnetic nanoparticles and chitosan [63]. Hemoglobin (Hb) was successfully immobilized on the surface of MCMS modified GCE with the cross-linking of glutaraldehyde. And another work reported that planar microcoils and distortion immune bridge measurement allow sensitivity of 8.4 pV/ Magnetic nanoparticles [64].Giant magnetoimpedance (GMI)-based biosensing system can achieve the targeted detection of cancer cells [65 ,66]. As show in Fig. 5, is the Scheme of the GMI-based detection system.

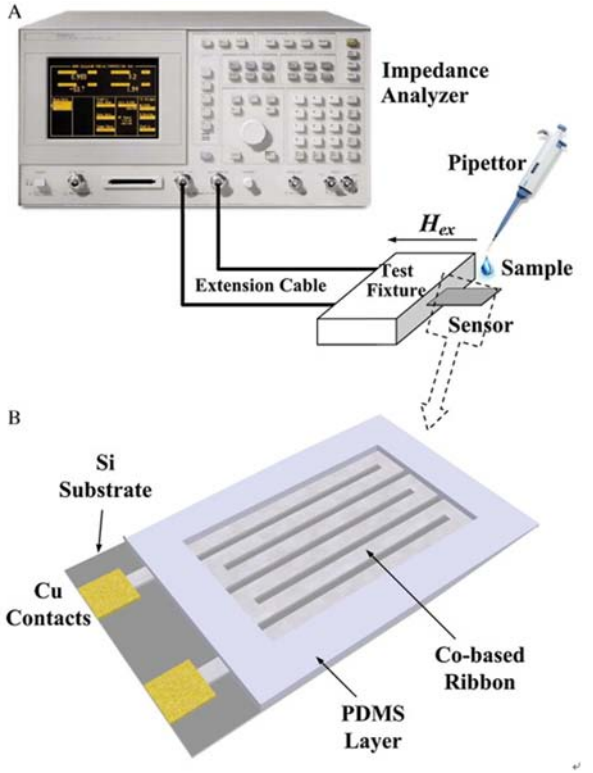

Fig. 5 Scheme of GMI-based detection system: (A) the biosensing system is consist of GMI sensor, a poly dimethylsiloxane (PDMS) mold, an impedance analyzer and a pair of Helmholtz coil; (B) partial magnification of the GMI sensing element.

3.2 Electrochemical method

An electrochemical biosensor is a biosensor with an electrochemical transducer. It is considered a chemically modified electrode (CME) since electronic conducting, semi-conducting or ionic conducting material is coated with a biochemical film [67]. Electrochemical DNA biosensors provide an effective alternative for genetically modified organisms' detection with their advantages of simplicity, low cost, and ease of use [68]. A novel electrochemical DNA biosensor based on zinc oxide (ZnO) nanoparticles and multi-walled carbon nanotubes (MWNTs) for DNA immobilization and enhanced hybridization detection is presented [69]. The obtained chitosan–ionic liquid–horseradish peroxidase biocomposite film possessed a porous morphology with orderly three-dimensional network, which possessed high electrocatalytic activity and fast amperometric response to H_2O_2.

Elena E et al [70]. reported the first application of an available RNA aptamer as the biorecognition unit as an electrochemical sensor for theophylline. Conformational changes of the aptamer upon binding of the ligand lead to

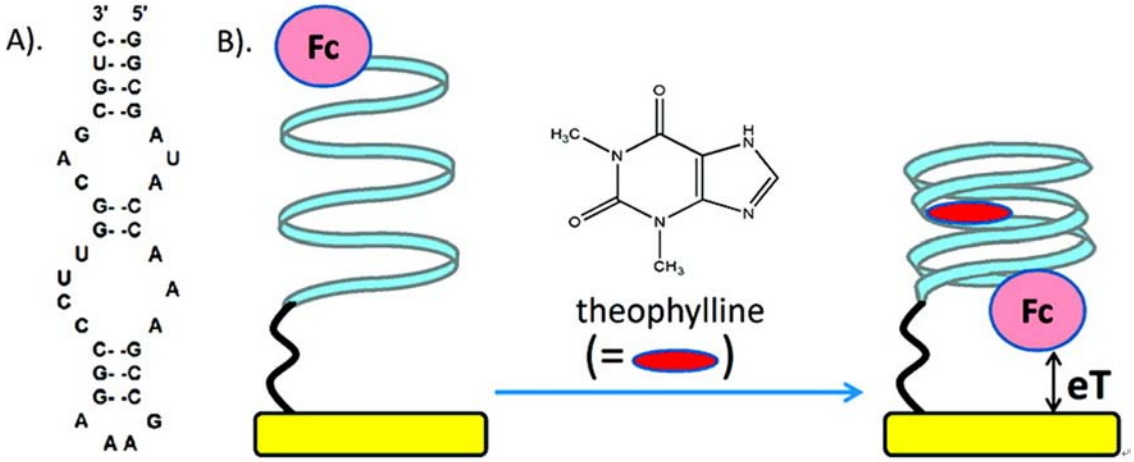

Fig. 6 (a) Theophylline-binding RNA aptamer sequence and **(b)** schematic representation of the electrochemical RNA aptamer-based sensor for theophylline (Fc) ferrocene)[68].

a change in either the efficiency of electron transfer (eT) to a redox probe conjugated to the immobilized aptamer or the interfacial eT resistance.

Mingli Yang et al [71]. attempted to use titanate nanotubes (TNTs) as the direct electron transfer promoter to develop a reagentless electrochemical biosensor for lactate detection. Lactate oxidase (LOx) enzyme has been immobilized on the three-dimensional porous TNT network-- offer the pathway for direct electron transfer between the electrode surface and the active redox centers of LOx.

3.3 Immune method

As we reviewed at before, gold nanoparticle is widely used in biosensor, especially in immunosensor. Rong Chai et al [72]. assembled gold nanoparticle and MB LBL into films on the GCE modified by thiourea for detection of HCG. Thiourea has two amino groups that the one can be immobilized onto surface of the pretreated GCE and the other can couple gold nanoparticles. Fig. 7 Showed the Schematic illustration of the stepwise immunosensor fabrication process.

In a report, microbeads are used to filter and immobilize antibodies and an immuno-gold silver staining (IGSS) method is used to amplify electrical signals that correspond to the bound antibodies [73]. The chip used for this system is composed of an inexpensive and biocompatible polydimethylsiloxane layer over a Pyrex glass substrate that contains a Pt microelectrode. Fig. 8 showed the analytic concept for the immunoassay. A new concept for coupling an inhibition biosensor immunoassay screening of low molecular weight analytes with nano-liquid-chromatography electrospray ionization time-of-flight mass spectrometry for confirmation of identity using an affinity chip interface has been presented [74].

H.Y. Lee et al [75]. reported a self-organized functional

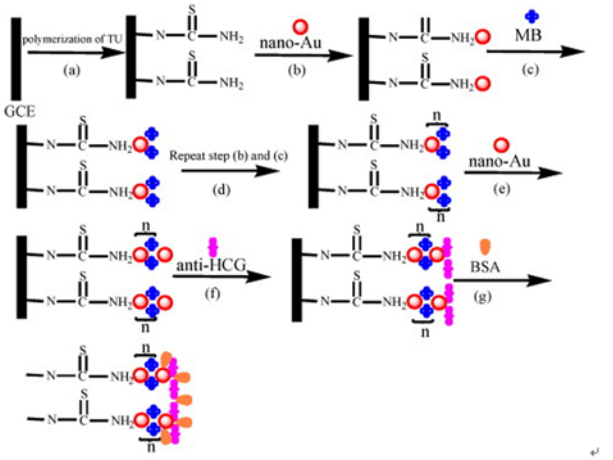

Fig.7 Schematic illustration of the stepwise immunosensor fabrication process: **(a)** successive CV scanning of TU film; **(b)** self-assembly of gold nanoparticle monolayer; **(c)** fabrication of {gold nanoparticle/MB} n multilayer films, n denotes the number of the layers; **(f)** anti-HCG loading; **(g)** blocking with BSA[70]

lipid vesicles (FLVs) array on nanometrics electrode using electrostatic interaction. The immobilized FLVs maintain their three dimensional configuration. The capture antibody can be immobilized firmly and apparently made to interact specifically with the target antigen.

3.4 Other methods

Shihong Chen et al [76]. proposed to immobilize enzyme for the fabrication of reagentless amperometric biosensor. After bounding thionine onto the BSA film, gold nanoparticle was adsorbed onto it to achieve gold nanoparticle/Th-BSA matrix for the immobilization of HRP and then possesses high enzymatic activity, affinity and low detection limit for H_2O_2. Ting Cao et al [77]. provided a versatile technique to fabricate chemically immobilized protein patterns by thin-film cracking. The pattern width can be varied from ~250 to ~2000 n. Covalently coupled antibody in the channel retains its specificity, and the antibody pattern can reach the saturated status in 10 min.

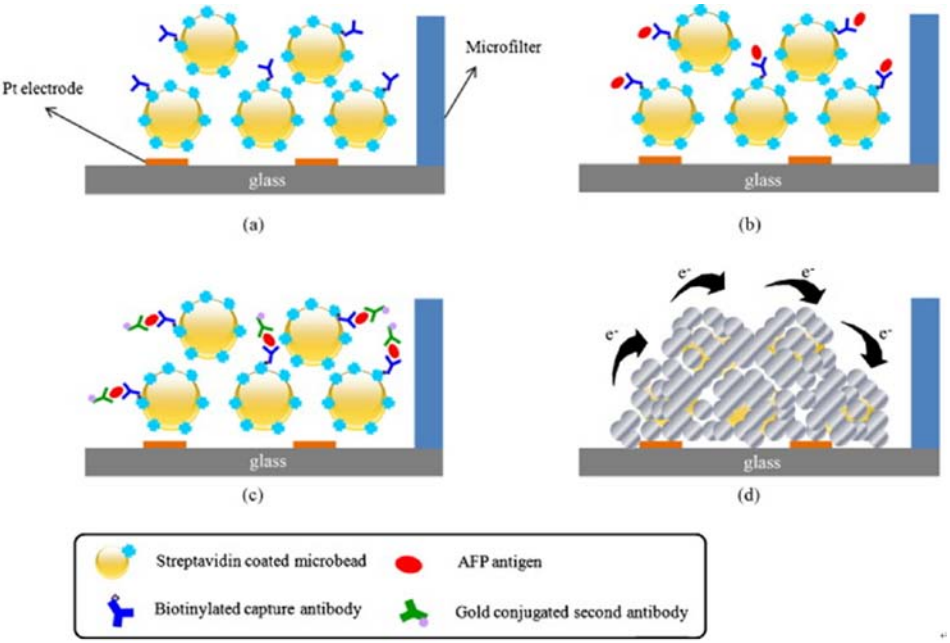

Fig. 8 Analytic concept for the electrical immunoassay using microbeads to facilitate the IGSS [72]

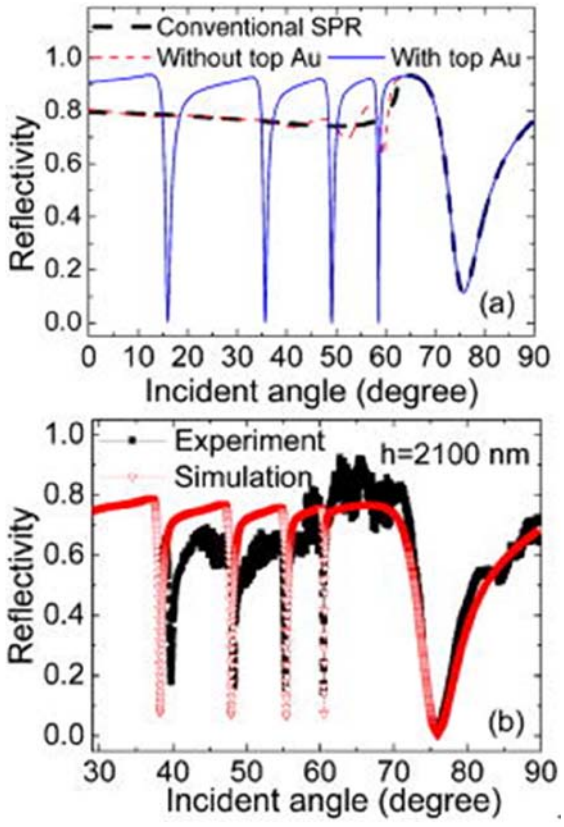

Fig. 9 (a) Comparison of the conventional SPR (dashed black line) and present detection method (blue). To illustrate the importance of the top gold layer, the reflectivity of a channel with bare glass (no gold layer) is also shown (dashed red line). The channel height is 1500 nm, bottom Au thickness is 40 nm, and top Au layer is 100 nm. **(b)** Measured and calculated resonance curves of the bulk optical modes [79]

Surface plasmon resonance (SPR) is an optical transduction mechanism that demonstrates extremely high sensitivity to surface binding events [78]. It is now regarded as one of the most sensitive direct non-labelled optical sensing techniques for real time monitoring of biological and chemical interactions. SPR images and SPR spectra could be obtained in analyzing protein arrays, because of the novel dual function SPR biosensor which is based on white light [79]. Elisabeth Galopin et al [80]. reported on a proof-of-concept system composed of a droplet based SPR system coupled to a surface acoustic wave microfluidic plateform. It is established that surface based binding analyses are highly influenced by the transport of analyte to the sensing surface. The SPR mode with many bulk modes, could be created in a microfluidic channel with metallic coatings on top and bottom [81] .The bulk modes are bulk sensitive and detect analytes in the channel with 20 times higher sensitivity than the SPR, which is particularly suitable for microfluidic devices (Fig. 9).

4. Applications of nanobiosensors

Biosensors have been applied in many fields and the most biosensors are developed for the detection of glucose, special DNA sequence, protein, pesticide and other small molecules. These nanobiosensors have common properties such as high selectivity, sensitivity and accuracy, rapid response.

4.1 Nanobiosensor for the detection of glucose

Since biosensors allow a wide range of transduction technology to be used, they have advantage over ordinary chemical sensors. Among them, the glucose biosensor has been widely used as a clinical indicator of diabetes and in the food industry for quality control. Due to its active surface, electrical properties and non-stoichiometric nature, nanostructured tin oxide plays an important role

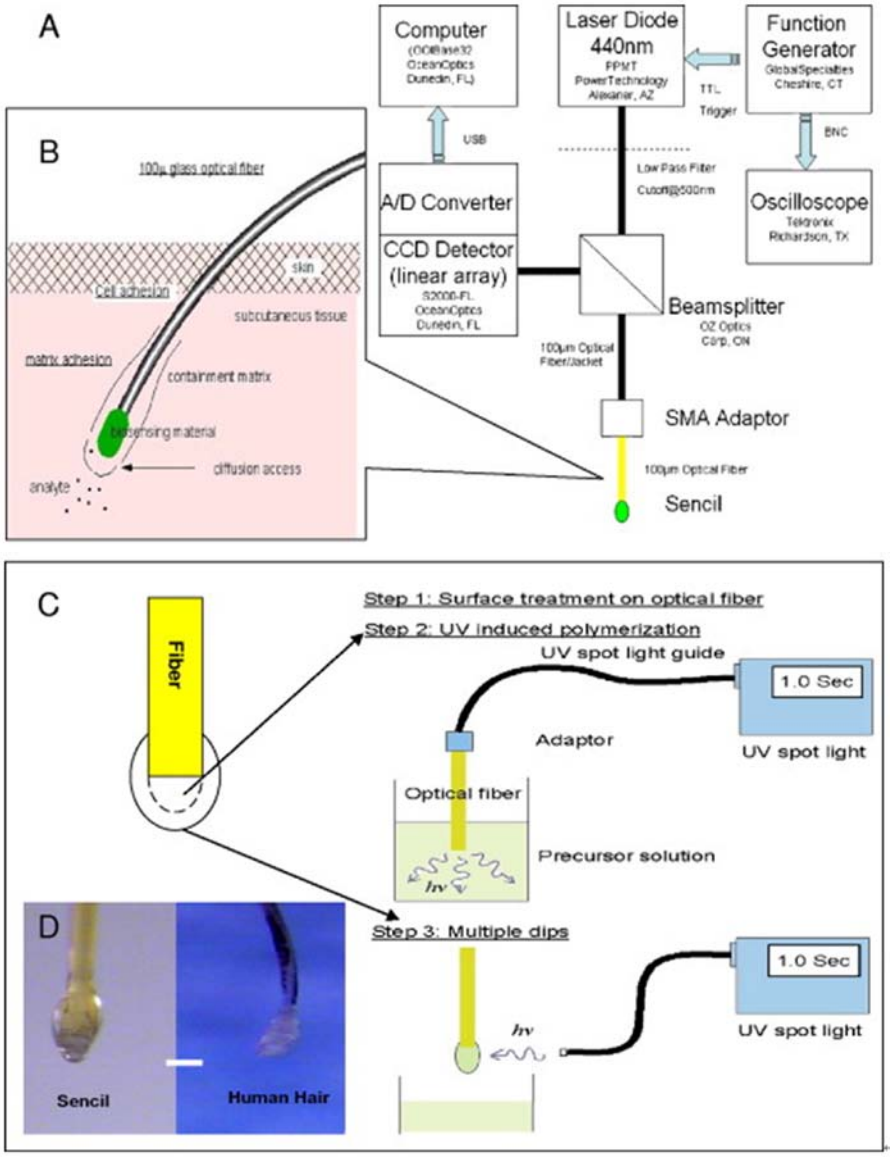

Fig. 10 Basic components and fabrication of Sencil prototype. **(a)** Laboratory spectroscopic instrumentation used to test prototypes; similar functionality must be miniaturized for portable clinical reader. **(b)** Sensor components and relationships to tissue in vivo. **(c)** Scheme of manufacture method with adhesion enhancement. **(d)** Similarity of shape and size between Sencil and human hair with attached follicle (white bar = 100 µm)[86].

in glucose sensor performance [82] .Bo-Wen Lu et al [83]. introduced an approach for fabrication of glucose biosensor based on drop-coating glucose oxidase on screen-printed carbon electrodes modified with Ferri-Nano-Fe_3O_4 mixture. And glucose biosensor with PQQ-GDH/ferricyanide/CNTs on a screen-printed carbon paste electrode exhibited a sensitivity of 31.0 µA mM^{-1} cm^{-2} to glucose with a linear range of 1–35 mM as well as only 2 µl volume and 20 s to response [84] . The mediator ferricyanide realized the electron transfer between the enzyme and electrode surface.

Fibrous morphology and wrapping of PDDA over MWCNTs result in a high loading of GOx into the electrospun matrix [85]. Pt nanoparticles could be electrodeposited on MWNTs matrix in a simple and robust way. The immobilization of glucose oxidase onto Pt/MWNTs electrode surfaces also could be carried out by chitosan-SiO_2 gel. The good results may be attributed to the synergistic action of Pt and MWNTs and the good biocompatibility of chitosan-SiO_2 sol–gel [86]. J.J YU et al [87]. fabricated a glucose biosensor based on GOx immobilized in Pt/CMK-3 matrix. With an extra Nafion film to eliminate the interferents, the resulting biosensor could be used to determine the glucose levels of serum samples.

S.G. Ansari et al. are developing a family of fiber-optic sensors called Sencils™ (*sensory cilia*), which can provide in vivo monitoring of various analytes for several weeks. The key element is a chronically implanted optical fiber (Fig. 10) with size and flexibility similar to a human hair and perfect fiber communication technology.[88] .And the illustration of glucose assay system is shown in Fig. 11.

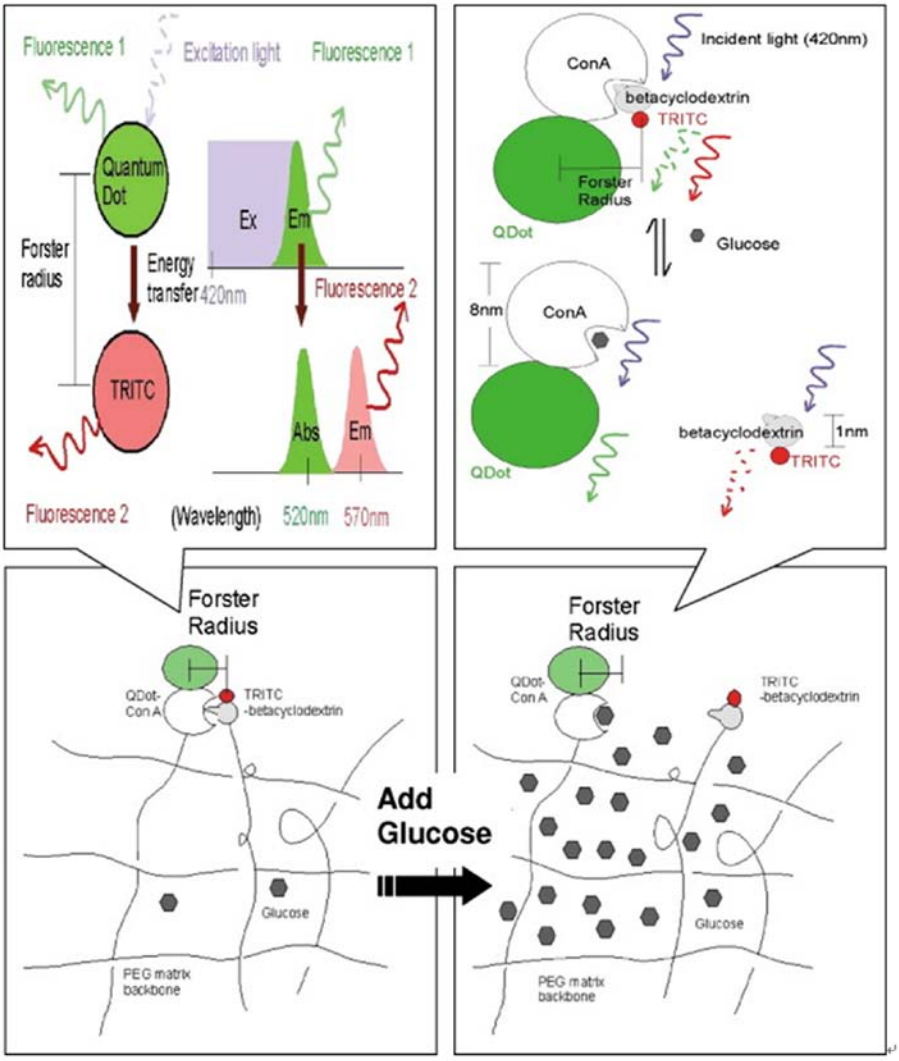

Fig. 11 Illustration of glucose assay system. Changes in FRET between fluorophores covalently immobilized on the flexible PEG matrix depend on changes in the distance between them, which in turn depends on the competitive natural affinity between Con A and various saccharides such as betacyclodextrin and glucose [86].

The classical method of glucose sensing involves the monitoring of H_2O_2 production by amperometric measurements. However, the requirement of a high positive potential (~0.6 V vs. Ag/AgCl) in this method leads to interference with other chemical species that influence the precise determination of the glucose concentration. Akira Kusakari et al [89]. demonstrated ODTA/PB/GOx LB films show a typical glucose sensor action at the potential of 0.0 V (vs. Ag/AgCl)(Fig. 12).

4.2 Nanobiosensor for the detection of DNA and protei

Sequencing of the human genome opened the way to the exploration of the proteome and this has lead to the identification of large numbers of proteins in complex biological samples. The solution of many of the technical challenges in proteomics and protein based molecular diagnostics will be found in new applications of nonmaterial [90]. Microcantilevers convert intermolecular reaction forces to detectable cantilever deflection in nanometers. Shi H et al [91]. first highlight the concept of coordinated nanobiosensors, which integrate desirable properties of the individual components: protein machinery for sensitivity and specificity of binding, peptide or nucleic acid chemistry for aligning the various electron-transducing units and the nanoelectrodes for enhancing sensitivity. Min Yue et al [92]. demonstrated label-free antibody-antigen binding assays by optically detecting nanoscale motions of two-dimensional arrays of microcantilever beams. Prostate specific antigen was assayed using antibodies covalently bound to one surface of the cantilevers by two different surface chemistries.

A biosensor for the detection of deep DNA damage is designed employing the bionanocomposite layer of MWNT in chitosan deposited on a SPCE [93].The biocomponent represented by double-stranded herring sperm DNA was immobilized on this composite using layer-by-layer coverage to form a robust film. Another biosensor employing immobilized DNA on a nano-structured conductive polymer fixed onto a platinum

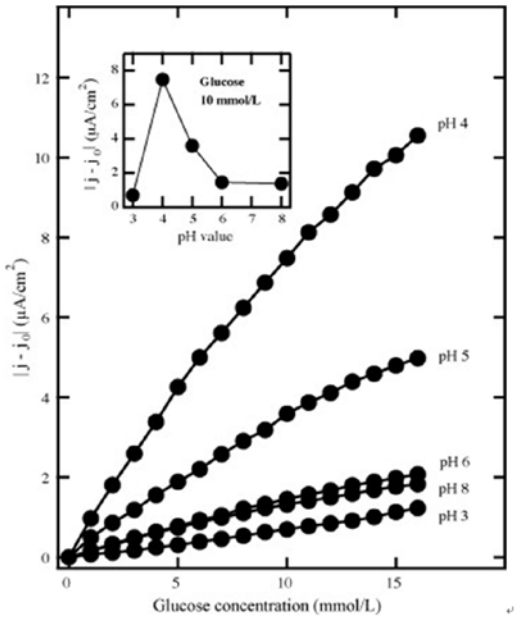

Fig. 12 Plots of response current density versus glucose concentration for the LB films (6 layers) prepared at different pH GOx solutions. The inset shows the current density at the fixed glucose solution of 10 mmol/L as a function of the pH [87].

electrode is presented as a new electrochemical sensor. Polypyrrole nanofibers, 30-90 nm in diameter, were synthesized in an aqueous media by the electropolymerization of pyrrole using normal PV. Double stranded DNA was physisorbed onto the PPy nanofiber films [94]. And for single-stranded DNA, gold nanoparticles functionalized with alkanethiol-capped LNA/DNA chimeras in a tail-to-tail hybridization mode could perform excellent [95]. and these probes show remarkable discrimination between a complementary target and one containing a single-base mismatch. Nano-SiO_2/p-aminothiophenol (PATP) film was fabricated by self-assembly and electrodeposition methods and was successfully applied to the detection of the PAT gene sequences by a label-free EIS method [96]. Wei Zhang et al [97]. fabricated a MWNTs/nano ZnO/chitosan composite film modified GCE and immobilized DNA probes on the electrode surface. The sensor can effectively discriminate different DNA sequences related to PAT gene in the transgenic corn, with a detection limit of 2.8×10^{-12} mol/L of target sequence.

4.3 Other applications of nanobiosensors

The liposome-based nanobiosensor could be used for the detection of organophosphorus pesticides. Porins embedded into the lipid membrane allow for the free substrate and pesticide transport into the liposomes. Pesticide concentrations down to 10^{-10} mol L^{-1} can be monitored using this inhibition fluorescent biosensor [98] . A work describes an approach for substrate integrating enzyme activation and corresponding current response at the substrate-bound enzyme electrode. Tyrosinase was activated by the use of reduced pyrroloquinoline quinone which was covalently bonded with the modified gold nanoparticles, which enhanced the sensitivity [99] .Another paper presented the chemistry of a deposited enzymatic layer (acethylcholinesterase(AChE) enzyme). The membrane is obtained by drying the gel in air flow and loosing of water molecules facilitates the enzyme accessibility to the support linking sites, by the interactions between the rest of polar and non-polar amino acids, ionic forces, hydrogen bridges and van der Waals forces [100].

Yuanbiao Qiao et al [101]. reported the facile electrochemistry of the Mb–$Zr(UMP)_2$•H_2O film on the GCE and the application to H_2O_2 biosensor. Porous nano-granule of zirconium uridine monophosphate, $Zr(UMP)_2$•H_2O is synthesized under mild experimental conditions and applied to the bioconjugation of myoglobin (Mb) to realize its direct electron transfer. The linear range for H_2O_2 detection is estimated to be 3.92–180.14 μM.

Hongying Zhu et al [102]. developed an optical sensor for on-line virus detection based on the opto-fluidic ring resonator has been developed. Microfabricated waveguides will be employed in replacement of the fiber taper to realize the microarray chip for detecting and analyzing multiple viruses in a parallel manner. Colin D. Medley et al [103]. developed a colorimetric assay for the direct detection of diseased cells. This assay uses aptamer-conjugated gold nanoparticles to combine the selectivity and affinity of aptamers and the spectroscopic advantages of gold nanoparticles. Samples with diseased cells present exhibited a distinct color change while nontarget samples did notas shown in Fig. 13.

Mitochondrial oxidative stress has been hypothesized as one of the earliest insults in diabetes. Some data support the hypothesis that the induction of MOS is more sensitive to hyperglycemia than the induction of the antioxidant response element (ARE). Delivery of an ARE-GFP construct with nanoparticles to the eye was successful using sub-retinal injection [104]. These data support the use of nanoparticle-delivered biosensors for monitoring the oxidative status of tissues in vivo.

Bingling Li et al [105]. reported an electrochemical aptasensor for adenosine based on impedance spectroscopy measurement, which gives not only a label-free but also a reusable platform to make the detection of small molecules simple and convenient. The method proposed did not rely on the molecule size or the conformational change of the aptamer.

The structure of Photonic crystals(PhC) depicted in Fig. 13,which is patterned using electron beam lithography on a siliconon-insulator wafer with a Si slab thickness of 400 nm. It also has shown that a Si 2-D PhCbased sensor can be used for detecting single particles that have a diameter

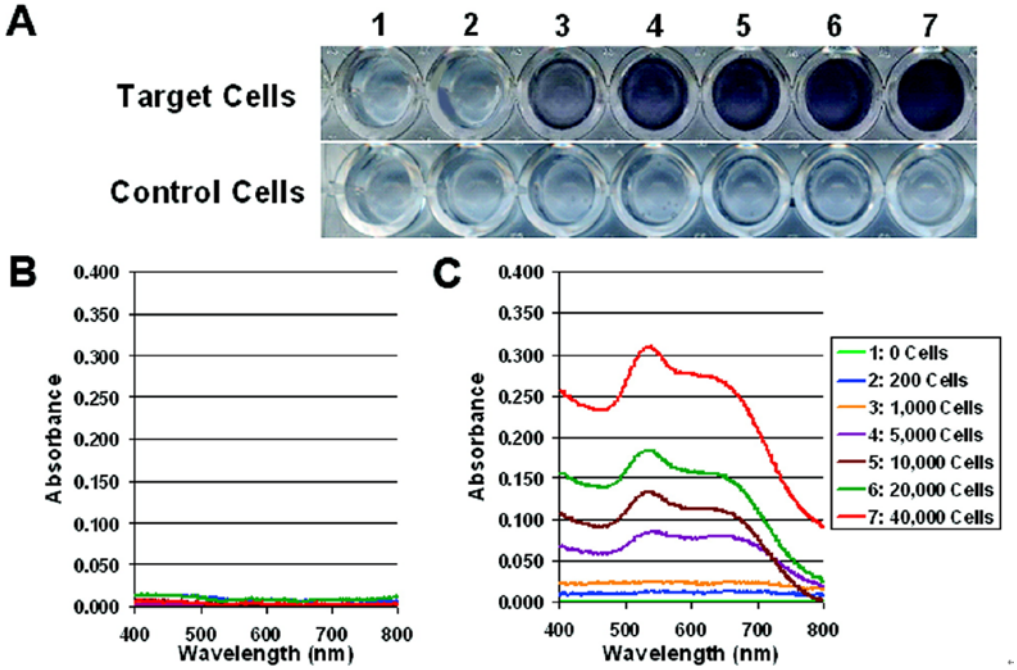

Fig. 13 (a) Images of ACGNPS with increasing amounts of target (top) and control cells (bottom). The amount of cells used in each sample is given in the legend on the bottom right. **(b)** Absorption spectra of the control cell samples with ACGNPs in Figure A. **(c)** Absorption spectra of the target samples with ACGNPs in Figure A [101].

of <50 nm. After proper functionalization, the device should be able to detect single viral pathogens such as severe acute respiratory syndrome [106].

5. Conclusions

The interdisciplinary cross of nanotechnology and biosciences opens the possibility for a wide variety of biological applications. Nanomaterials have shown numerous unique physical, chemical, electronic, and optical properties. We believe that nanotechnology used to biosensor will improve biomedical diagnostics and therapy.

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