Advanced Review

Nanoprobes for enhanced electrochemical DNA sensors

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Biosensors, small devices enabling selective bioanalysis because of properly assembled biological recognition molecules, represent the fortuitous results of years of interdisciplinary and complementary investigations in different fields of science. The ultimate role of a biosensor is to provide coupling between the recognition element and the analyte of interest, bringing a quantitative value of its concentrations into a complex sample matrix. They offer many advantages. Among them, portability, low cost with fast response times, and the possibility to operate *in situ* without the need for sample preparation are certainly the most important. Among biosensors, a large space is occupied by DNA biosensors. Screening genomic DNA is of fundamental importance for the development of new tools available to physicians during the clinical process. Sequencing of individual human genomes, accomplished principally by microarrays with optical detection, is complex and expensive for current clinical protocols. Efforts in research are focused on simplifying and reducing the cost of DNA biosensors. For this purpose, other transduction techniques are under study to make more portable and affordable DNA biosensors. Compared with traditional optical detection tools, electrochemical methods allow the same sensitivity and specificity but are less expensive and less labor intensive. Scalability of electrochemical devices makes it possible to use the advantages introduced by nanosized components. The involvement of nanomaterials and nanostructures with custom-tailored shapes and properties is expected to rapidly boost the field of electrochemical DNA biosensors and, in general, that of next-generation sequencing technologies.

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INTRODUCTION

In the last decade, DNA arrays have attracted increasing attention, with acceptance for a broad range of applications. These devices are relevant for the diagnosis of genetic diseases, detection of infectious agents, study of genetic predispositions, development of personalized medicine, detection of differential genetic expression, forensic science, food safety investigations, and environmental monitoring.[1–5] DNA arrays offer the possibility of screening large selections of genes at the same time, on the same device and with a short response time. These advantages make them relevant analytical tools that have been commercialized by different companies: Affymetrix, Illumnia, Agilent Technologies, Roche NimbleGen, and others. All these devices use a fluorescence marker for hybridization detection.

As an alternative to the microarray technology based on fluorescent labels and optical detection systems, electrochemical analysis offers the same sensitivity but with cheaper and scalable devices. For these reasons, much effort has been spent in

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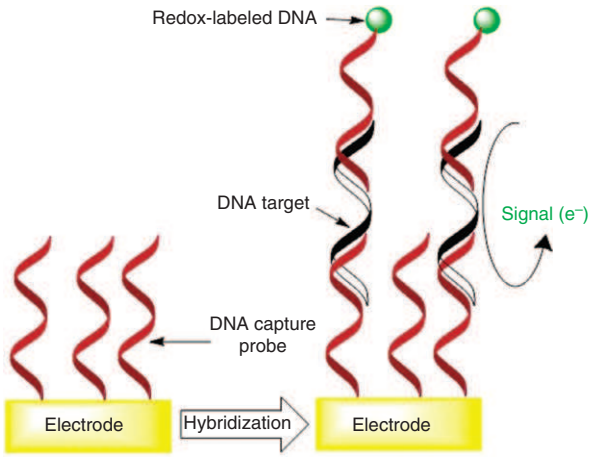

FIGURE 1 | Scheme of electrochemical DNA biosensor with a sandwich format.

developing an electrochemical biosensor for DNA analysis.[6] The first attempts replaced the optical markers by electrochemical redox molecules, such as ferrocene or redox enzymes (horseradish peroxidase, alkaline phosphatase), but with similar sandwich configurations[7] (Figure 1).

Progress in the study of the interaction of DNA with small molecules able to intercalate along its helix structure has led to the introduction of redox indicators for application in DNA biosensors. They divide into[8] intercalating molecules that can insert adjacent base pairs and groove binders[9] that can bind to the groove of DNA. Organic dyes and metal complexes are among the latter. In particular, Methylene Blue, Deunomycin, Hoechst 33258, $Co(phen)_3^{3+}$, Echinomycin, and $Ru(bpy)_3^{2+}$ are examples of electrochemically active DNA ligands explored in DNA detection applications.[10] Seminal studies in this field were started by Berg.[11] Platforms based on the use of redox-active markers bound at the target (or probe) surface used to rely on monitoring the current peaks produced by these intercalated redox species, under proper selection of the electrodes and of the pH of the electrolyte. Their efficiency for DNA analysis is intimately related to the properties of the marker used, like reversible electron transfer at low potential, and the ability to differentiate between ssDNA (probe) and dsDNA (target probe).

The unique properties of molecular beacon probes have also been explored as a substitute for standard ordinary probes in electrochemical DNA biosensors. A biosensor with a picomolar limit of detection (LOD) was obtained through the conformational change that characterizes molecular beacons at hybridization[12] in a platform that uses ferrocene as the electroactive label (Figure 2). Here, hybridization

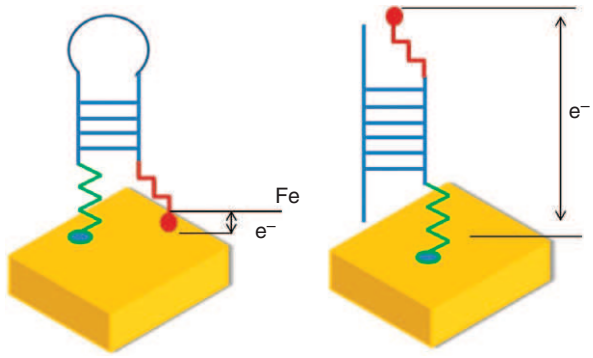

FIGURE 2 | The E-DNA sensor of Fan et al.[12] The onset of hybridization is followed by a rapid change in the redox current that is attributed to the increased separation of the redox label by the gold electrode surface.

is followed by the spontaneous disruption of the stem–loop to a rigid rod-like structure in which the ferrocene is found at a larger distance from the electrode surface.

The discovery of electroactivity of the DNA molecule, in the early 60s, opened the possibility of using electrochemical analysis as a reliable transduction system for hybridization detection without the need for redox markers. The first attempts based on the direct electrochemistry of nucleic acids showed their limitations quite early; the signals coming from the redox guanine bases, contained in the DNA sequence, were not only irreversible but also obtained at highly applied potential, where the associated background compromises clear detection. To improve the signal-to-noise ratio, modifications of the probe sequence, by replacement of guanine with ionosine, which oxidizes at different potentials, was considered, but similar results were achieved. In the early 80s, the introduction of DNA-modified electrodes boosted the field of electrochemical DNA biosensors.[13–16] This methodology, refined by Palecek's group, soon became popular. Based on the strong adsorption of DNA at metallic surfaces, the mercury (or carbon) electrode is easily DNA-modified just by short periods of immersion in a solution containing nucleic acids. Modified electrodes, measured by adsorptive stripping analysis, increased the detection sensitivity by several orders of magnitudes and reduced the sample volumes of previous platforms by approximately 2–3 orders.

Also in the 60s,[17] it was postulated that the electronic system of the π-stacked bases of DNA supported charge transfer (CT) over long distances. About 30 years later, the hypothesis was confirmed by the observation that in the presence of DNA, the rate for photo-induced electron transfer was two orders of magnitude faster.[18,19] Although the mechanisms of DNA-mediated CT are still debated, its

chemistry is very sensitive to different variations in DNA base stacking, and this property could explain its ability to repair proteins that quickly localize a single point mutation over the entire genome.[20] Alterations of the π-stacked pairing cause attenuation of the electrochemical current and provide a direct method to detect single base mismatches.[21] Electrochemical DNA hybridization sensors based on long-range electron transfer from a redox intercalator through double-stranded DNA have also been developed.[22,23] In these platforms, two anionic intercalators, 6-anthraquinone di-sulphonic acid and 2-anthraquinone mono-sulphonic acid, have successfully been used as redox indicators with selectivity for one mismatch in a mixture of DNA targets. The dropping current observed in cases of mismatched targets confirms that alterations to the π-stack inhibit CT.

The field of electrochemical DNA biosensors has rapidly grown, but it suffers from drawbacks that limit the application of discussed devices to real-life tasks. The recent availability of different types of nanostructures and nanomaterials is promising to overcome difficulties in current technologies.

NANOSCALE: ADVANTAGES OF ELECTROCHEMICAL DNA BIOSENSORS

The emergence of materials and structures at the nanoscale should radically improve the current state of the art of electrochemical DNA analysis. Nanoprobes and nanomaterials, with extraordinary high surface-area-to-volume ratios, are very attractive for use in this field. Scaling down the device size to fit that of the molecules to analyze intrinsically enhances the efficiency of the coupling between the biomolecules and the probes used for their detection. New phenomenon emerging at the nanoscale can also be utilized and have the potential to reach single-molecule detection sensitivity.

According to a simple, analytical, solvable diffusion-capture model that describes the kinetics of the absorption of biomolecules at the surface of a nanosensor in the presence of a solvation effect,[24] the performance limits of nanoscaled biosensors can be easily determined. By comparing three possible geometries that can arise, ranging from the two-dimensional planar geometry to the three-dimensional spherical one (see Figure 3), it is concluded that the most highly active areas lead to higher detection limits, which are theoretically able to reach aM concentrations of the target.

In the following section, we introduce some of the electrochemically based nanodevices for DNA analysis that have been developed to date.

NANOWIRE AND NANOTUBE DNA SENSORS

One-dimensional nanowires (NWs) and nanotubes (NTs) are particularly interesting for applications in biosensors for their extraordinary surface-area-to-volume ratio and for their compatibility with electrical detection systems. Despite having nanometric diameters, they can be up to centimeters long, with length-to-diameter aspect ratios exceeding 10.[7] In the case of NWs, such as silicon NanoWires (SiNW), it is currently possible to control the growth process, and both the electrical and the optical properties can be adequately custom tuned. In biosensors, NW and NT are usually arranged in a field-effect transistor (FET) geometry in which they connect the two metallic electrodes, the source and the drain where currents are injected and collected. In traditional FETs, the flux of charges is controlled by the action of a third electrode, the gate at which the potential is applied. According to the sign of the gate potential, the charges are depleted or accumulated in such a way to produce the change of conductance that turns on/off the electronic device. This makes the FET an ideal candidate for biosensors. The gate semiconductor is functionalized with molecular receptors, which selectively bind to the analyte of interest so that the target molecule itself acts as an electrical gate for its own detection.

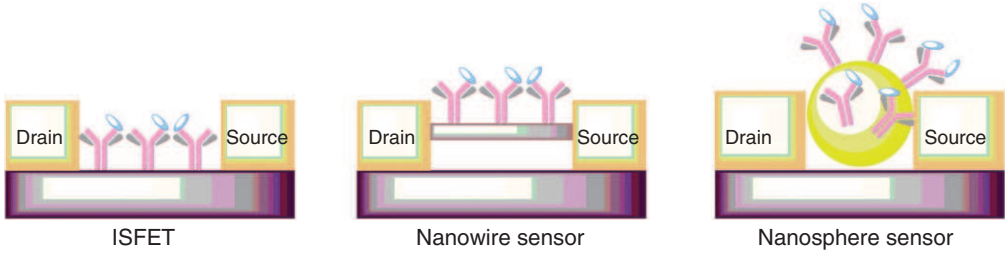

FIGURE 3 | The different nanosensor geometries considered.[24]

NW Sensors Based on Carbon Nanotubes

Carbon nanotubes (CNTs) can be thought of as narrow graphene sheets rolled to form a continuous tube. Compared with metals, CNTs have higher melting points and are stronger against applied tension. The high degree of atomic-scale perfection makes them chemically inert. They arise in two forms: the single-walled nanotube (SWCNT) or multi-walled nanotube (MWCNT). The atomic structure and diameter size determine their electrical behavior, which can vary from that of a ballistic conductor to that of a semiconductor.

A CNT-FET is constituted by a CNT embedded between two metallic electrodes in a dielectric, a top gate and a ground plane, used as a source and a drain.[25] Compared with conventional semiconductor materials, CNT-FETs have superior electrical characteristics, higher charge mobility and chemical inertness.

The scarce carrier density charge of SWCNTs, comparable with that of proteins at the biosensor surface, makes SWCNTs particularly well suited for electrical detection assays. ssDNA strongly interacts with the SWCNT: just a few hundred picoseconds are required to bind ssDNA to the external surface of uncharged, or positively charged, SWCNT.[26] This is due to the exposed hydrophobic sites that can easily interact with the hydrophobic (graphene) surface of SWNT.

The employment of CNT-FET as an electrically based biosensor for detecting DNA hybridization follows the general scheme introduced before: hybridization at the gate surface of a CNT-FET induces a negative charge that changes the conductance on the CNT and triggers the FET.

An electronic SWCNT biosensor that can effectively detect two random synthetic DNA sequences 15 mer and 30 mer in length has been proposed by Tang et al..[27] In this device, a network of approximately 100 SWCNT, grown almost parallel between the electrodes, and a platinum wire inserted in the buffer solution, are used as the top gate electrode. Thiolated ssDNA is anchored to the surface of gold electrodes. The change in charge density, following the hybridization of complementary DNA, enables DNA hybridization detection. Addition of a complementary cDNA is followed by a decrease of about the 25% of the conductance. On the other side, no differences are seen in the control assay in which only PBS and the mismatched sequence are added to the device surface.

Another CNT-FET biosensor for the electrical detection of DNA hybridization, real-time and label-free, based on the use of a SAM of peptide nucleic acid (PNA) probes has been developed.[28] PNAs, a synthetic polymer in which the phosphates and deoxyriboses of the DNA backbone are replaced by a polypeptide, are frequently exploited in genetic sensors. PNAs provide better stability with respect to the ionic strength and improved resistance to degradation by proteases and nucleases; they are also electrically neutral with improved hybridization properties compared with DNA, due to the lack of charge repulsion. The high synthesis costs of PNA, on the other hand, limit their application. In this platform, the biomolecules are not directly immobilized on the SWNT surface but are self-assembled on a gold electrode coating on the backside of the device and connected to the gate electrode by means of a microfluidic channel through which complementary and mutated sequences are delivered. By monitoring the conductance change after delivery of the complementary target and mutated targets, detection of an 11-mer oligonucleotide complementary sequence was successful with an LOD of 6.8 fM.

Many studies have attempted to clarify the sensing mechanisms of CNT-FET, but a clear understanding is still lacking. Among the proposed mechanisms are the electrostatic gating, the change in the gate coupling, the changes in the carriers' mobility and the unconventional Schottky Barrier (SB) effect. Systematic study[29] aimed at elucidating this point individuated the electrostatic gating and/or SB effect as the leading sensing mechanisms. A similar conclusion has also been reached theoretically.[30]

NW Sensors Based on SiNW FET

FET nanosensors based on semiconducting silicon bridges allow an efficient CT between the surface-attached DNA and the NW. Contrary to the case of SWCNT, SiNW can be prepared by exploiting either 'bottom-up' or 'top-down' fabrication processes.

Unlike SWNCT, the electrical properties of SiNW are highly reproducible and can be tuned during the growth process, which is currently well controlled. Hahm and Lieber[31] demonstrated the effectiveness of a SiNW-based biosensor for the direct electrical detection of DNA mutations with ultrasensitive responsiveness. This biosensor used PNA as the capture probe (CP), and it was used to detect two different mutations of cystic fibrosis. The use of a PNA receptor increased the hybridization efficiency and helped to achieve hybridization at low ionic strength. The workability at low ionic strength is crucial in FETs because they respond to changes in the surface charge, and increased ionic strength comprises the electrical double layer around the wires.[32] By monitoring the conductance change at the SiNW surfaces,

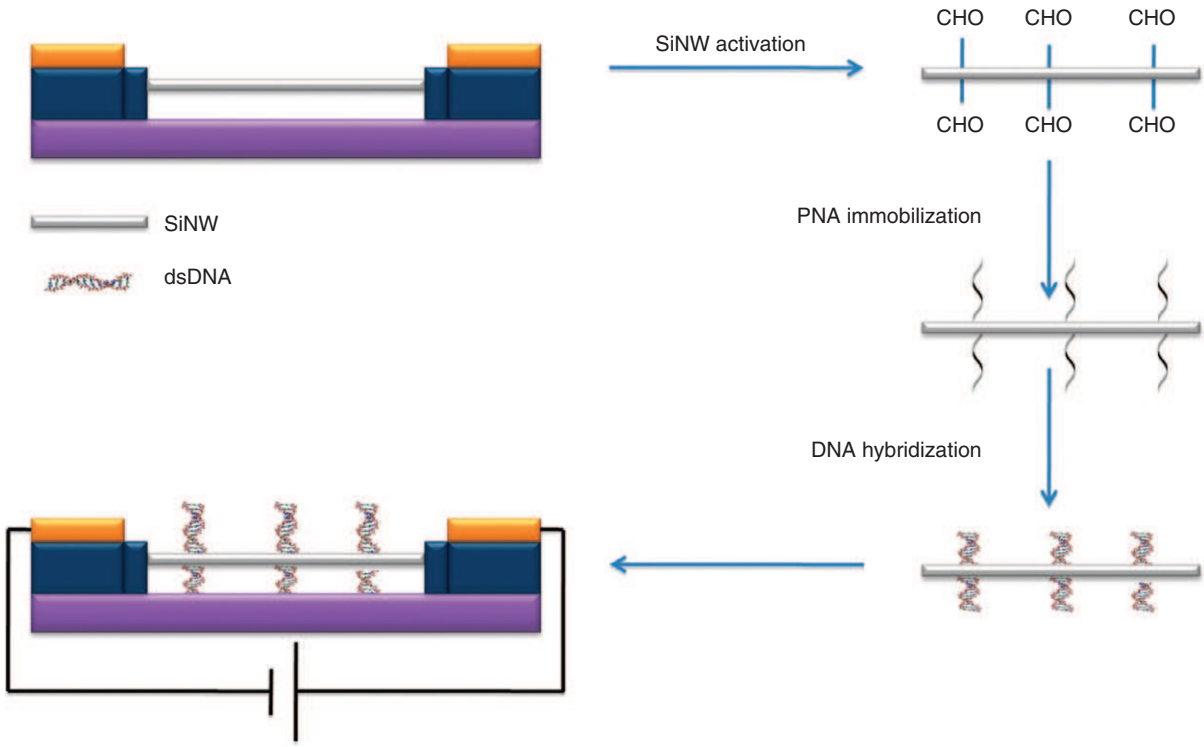

FIGURE 4 | Schematic of the SiNW-based sensor used for the ultrasensitive label-free electrical detection of DNA hybridization.[33]

this device detected fM concentrations of the analyte. Efficient and sensitive label-free electrical detection of DNA hybridization has been shown[33] by applying a reliable and scalable fabrication technique for producing uniform and well-aligned SiNW (Figure 4).

The device is created by combining deep ultraviolet lithography and self-limiting oxidization, which allows sensitivity down to fM concentrations. SiNW arrays were obtained on patterned and etched silicon-on-insulator (SOI) wafers, further submitted to oxidation, contact metal deposition, and passivation. Microfluidic channels, embedded on the SiNW arrays, provide isolation of all the electrical contacts from the aqueous solution. The SiNW were functionalized with PNA CPs via the silane chemistry. DNA detection was established by monitoring the resistance after immersion in the hybridization buffer with different concentrations of complementary DNA. The changes in the resistance observed are a consequence of DNA hybridization, which induces a decrease in the carrier concentrations on the surface of the n-type SiNW used, giving rise to a field effect that allows detection.

NW Sensors Based on Conductive Polymers

Of particular interest is the case of conductive polymer NWs made with polypyrrole (Ppy), polyaniline (PANI), and polythiophene. These wire polymers have been used in DNA hybridization sensors due to their ability to behave both as bio-receptor immobilization surfaces and as transduction agents at the same time.[34] However, their electrochemical, optical, and electronic properties are sensitive to environmental changes occurring at their surface, such as the ones produced in the DNA recognition process. In addition, there is a wide range of functionalization strategies available for conducting polymers that allow easy biological functionalization. This imparts the required selectivity of the sensor and provides an intimate contact between the DNA probe and the polymer, enhancing the stability and speed of the response.[35] In addition, the possibility of tuning the conductivity values over several orders of magnitude, just by changing the dopant, solvent, synthetic method, oxidation state, etc., together with the stability of the monomers under ambient conditions and their inexpensive cost make use of conducting polymers ideal alternatives to build NW-based DNA sensors.

Recent reports have proved the high sensitivity and stable performance of single conducting polymer NWs in comparison with other conducting polymer-based DNA sensors.[36,37] Bangar et al. revealed the strong impact of single NW devices of this type in medical diagnosis by describing a label-free DNA sensor based on single polypyrrole NWs[37] (Figure 5). They detected single nucleotide

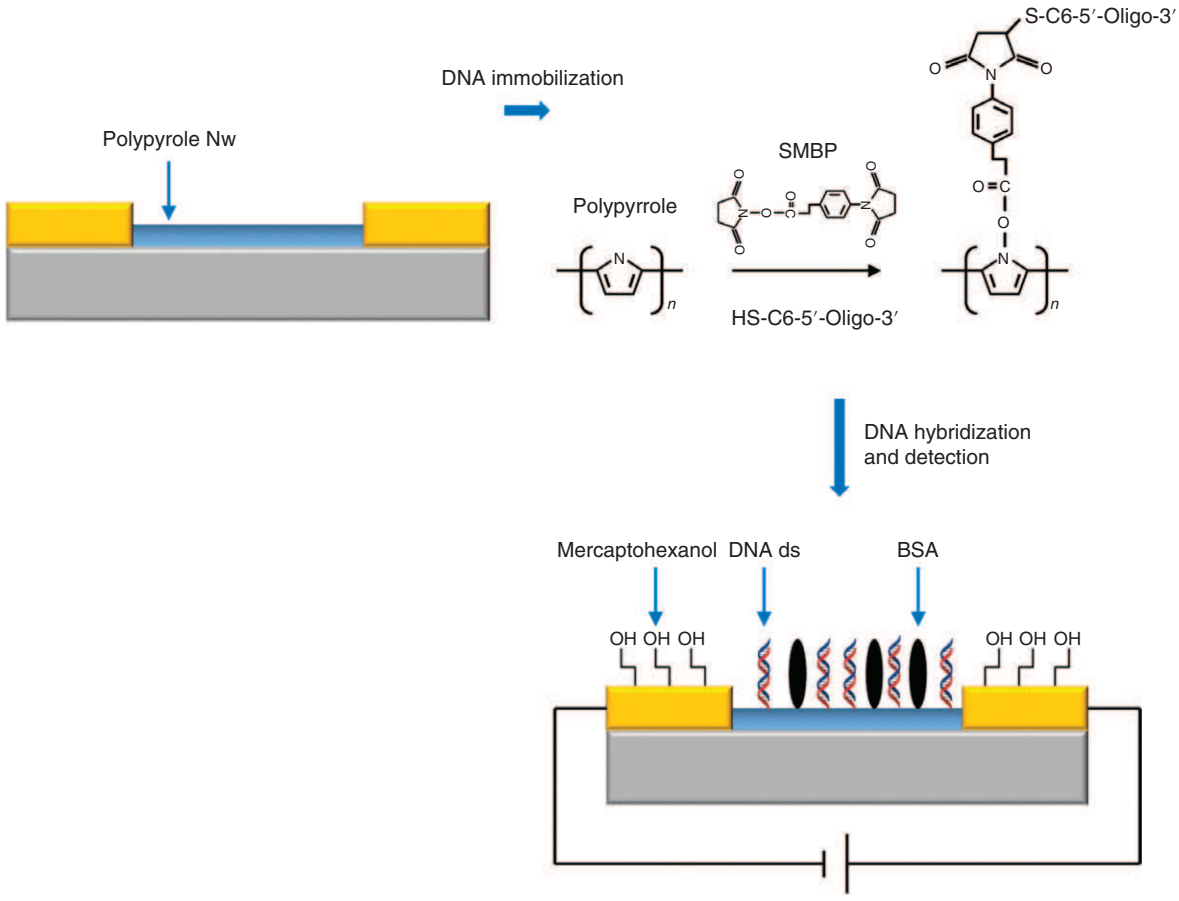

FIGURE 5 | Schematics of the polypyrrole NW-based sensor used in Ref 37 for the label-free DNA detection.

polymorphisms (SNPs) of breast cancer gene sequences in very small sample volumes (30 μL) with limits of detection as low as 10 fM. Such results demonstrate the great potential of single conducting polymer NW devices for DNA detection.

However, serious limitations are still found in their creation because conductive polymers are generally incompatible with the conventional fabrication techniques employed in the field of micro/nano electronics.[38] Alternative methods based on the synthesis of NWs in solution (by template methods)[39] have become the most popular approaches. Here, a suspension of NWs is dropped between the metallic electrodes, and single NWs are then aligned between the contacts by use of electric or magnetic fields.[40] Other approaches have used scanning probe techniques, such as scanning tunneling microscopy (STM), the atomic force microscopy (AFM) or the dip pen nanolithography (DPN), and others have attempted the *in situ* electrochemical polymerization of NWs between the electrical contacts.[41–43]

Nevertheless, all these techniques are time consuming, require tedious alignment steps and are not fully understood. The development of standard procedures to assemble these nanomaterials into functional sensor circuits is one of the remaining challenges in this field.

NANOSENSORS BASED ON GOLD NANOPARTICLES

Colloidal gold is the name used for the suspension of nanometric particles of gold in a fluid, usually water. GNPs have been an attractive material in research for a long time.[44] A practical advantage of GNPs is their shape, size, and physico-chemical properties that can be tailored by controlling the preparation parameters to provide an efficient coupling with different types of biomolecules.

The biocompatibility of GNPs provides a stable surface for the immobilization of the biomolecules that does not interfere with their ordinary biological activity. The well-explored conjugation chemistry at the gold surface makes it possible to realize efficient couplings between the biological recognition element and the surface. The change produced by the target

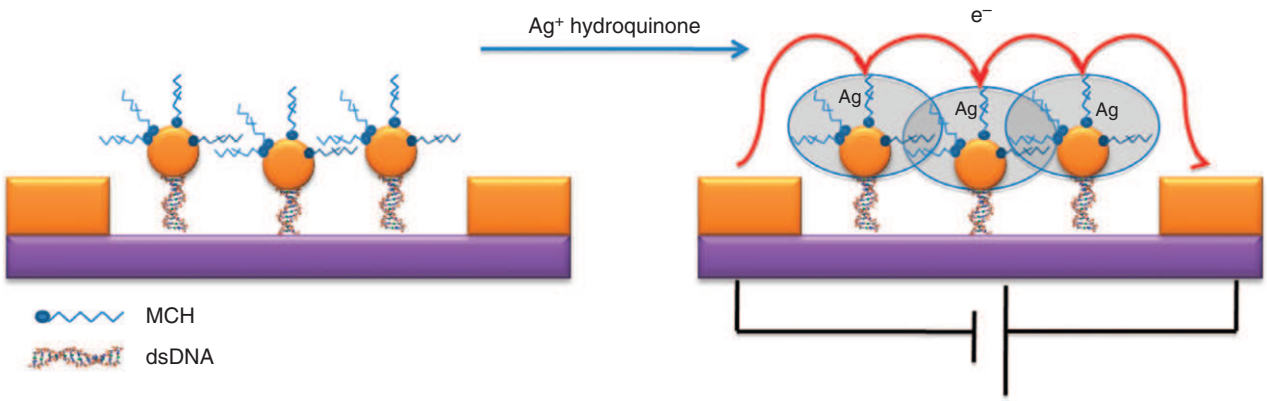

FIGURE 6 | Schematic of the standard protocol applied in silver enhancement treatment explored in many GNP-based nanosensors.

binding at the nanoparticle surface affects its optical and/or its electrical properties and can be usefully exploited in biosensor applications, as discussed in the following section. An electrical detection assay, which combines GNPs with a signal amplification strategy based on metal enhancement treatment, has been explored by different authors after the seminal work by Park.[45] In the presence of silver ions and hydraquinone, GNPs promote the selective, and catalytic, deposition of silver metal, which allows DNA hybridization detection with sensitivity ranging from the high aM to the mid-pM range.[46] In the device developed by Park and colleagues,[45] a short oligonucleotide CP was located between the microelectrode gap while a longer target oligonucleotide, with recognition elements complementary to the CP and labeled with GNPs, was provided. Thereafter, binding gold nanoparticles (GNPs) filled the microelectrode gap and underwent a silver-enhanced treatment with a photographic developing solution that uses GNPs to promote the reduction of the Ag (I) and hydroquinone and increased the sensitivity of the device. The detection of hybridization was then achieved by monitoring the gap resistances at increased time exposures to the silver enhancement treatment in the presence of complementary and mutated strands. The deposition of silver is turned on by the nanoparticles and thus by the hybridization event. In the control experiments carried out with denaturalized strands, no detectable signals were measured (Figure 6). Silver enhancement treatment has also been used in combination with stripping metal analysis and a discrimination strategy against not-hybridized DNA based on magnetic separation.[46]

The combination of silver/enhancement treatment with stripping metal analysis and the use of an efficient discrimination strategy against not-hybridized DNA relying on the magnetic separation were reported in Ref 45.

Here, a biotinylated DNA probe is bound to a magnetic latex sphere coated by streptavidin. The hybridization of the biotin-labeled DNA target is followed by the formation of the GNP-streptavidin couple and the catalytic silver precipitation on gold labels, which is detected by applying stripping metal analysis based on electrochemical potentiometric measurements. The removal of non-hybridized DNA is achieved by means of magnetic separation, which allows an efficient minimization of non-specific binding, thus increasing the efficiency of the hybridization response. With this technique, an LOD of 10 pg to 50 μg/L after a hybridization time of 20 min can be achieved, and lower detection limits are expected for longer hybridization times. Two different array formats, which also rely on the use of magnetic beads to trigger the direct electrochemical detection of the GNP tags on a magnetic graphite-epoxy composite electrode (M-GECE), have been explored.[47] Two alternative designs, a so-called *two strands assay format* and a *sandwich format*, have been proven to be effective in the detection of hybridization of the breast cancer gene BRCA1 and of the cystic fibrosis gene, respectively. In the former case, the CP is immobilized onto biotinylated paramagnetic beads; after immobilization, the hybridizing solution containing the target is added, and the whole molecular assembly is exposed to a solution containing streptavidin-coated GNPs. The detection is carried out by direct differential pulse voltammetry (DPV), through which the GNP tags present in the conjugate are measured.

A similar protocol applies to the case of the sandwich-based, alternative array design. In this format, a further hybridization step is necessary to conjugate the target probe, which is attached to the paramagnetic beads, to a second biotinylated probe for signaling with streptavidin-coated GNPs. In both cases, the device has great discrimination efficiency

in control experiments performed with a three-base mismatched sequence and with a non-complementary strand. Indeed, no current signal is observed in these cases, while a clear current DPV peak is reported for the complementary strands. The two strand assay formats reach a sensitivity of 600 nM.

NANOSENSORS BASED ON DNA WIRES

The last example of nanosensors for DNA detection addresses to the formation of the NW, which links the source and the drain of two electrodes with the DNA molecule itself.

Both electrodes are functionalized with DNA bioreceptors specific to different DNA target regions. These gold functionalized electrodes are separated by an insulating thickness (usually air) of nanometric size. The complementary DNA target is trapped inside the electrodes and analyzed by monitoring the electrical properties of the nanogap. Apart from their usability in biosensing platforms, nanogap electrodes have been used to study charge transport mechanisms in biomolecules,[48–50] which plays a fundamental role in photosynthesis and it is thought to have implications in oxidative damage to DNA and its repair mechanisms.[51,52]

Planar,[53] as well as vertical[54] (Figure 7), nanogap electrodes have been used to date to develop DNA biosensors with the ability to detect SNPs. In both these biosensors, two gold electrodes are separated by a distance of approximately 60 nm. The two opposite sides of the nanogaps are functionalized with two short CPs, which are thiolated ssDNA chosen antisymmetric to each other, and in such a way to have the last 20 bases complementary to a target ssDNA. The size of the target DNA itself is adequate to fit that of the nanogaps. In the first platform, the hybridization of the target is followed by an incubation step with nuclease for the elimination of mismatched duplexes and free CP strands. In both platforms, the created DNA nanobridge is recovered with 1.4 nm GNPs to enable hybridization transduction. Detection is achieved by analyzing the current–voltage (I–V) curves across the nanogaps after the formation of a metal NW on the hybridized DNA. The analytical performance of this biosensor for SNP genotyping has been shown for the detection of KRAS protein's mutation, which is implicated in the development of many cancers. An LOD of 10 fM is reached after 30 min of incubation with the nuclease, followed by 10 min of exposure to the solution for silver metallization. Similar promising results are also obtained in the case of the VNG-based biosensor that has an LOD from 1.0 fM to 1.0 pM.

An improved design of the wire DNA sensor, involving facing the two electrodes in a short gap distance of a few nanometers (50 nm), enables DNA hybridization detection free from the metallization process.[55]

Here, the two antisymmetric molecular receptors, which are provided with a thiol terminal, are immobilized inside the vertical walls of a planar nanogap through the well-known sulfur–gold affinity. The non-active sensor area is blocked with SiO_2, just opening to the gold on the nanogap. The face-to-face distribution of the two electrodes permits the formation of straight DNA wires between the electrodes, which allows an ordered orientation of the π-stacked bounds of the DNA bases and improves the long-range electron transport through the DNA molecule robust enough against environmentally induced perturbations, and thus provides a direct tool to detect the molecule. The I–V characteristics of the nanogaps are measured after each stage of the fabrication and functionalization process. The clear increase in the nanogaps current, which is measured after the addition of the complementary target, shows the feasibility of this label-free strategy based on charge transport along DNA. To demonstrate the selectivity of the biosensor and to further assure that the charge transport is supported by the molecule, the device was tested with two mutated CPs. The weak current measured for this device, in agreement with previous

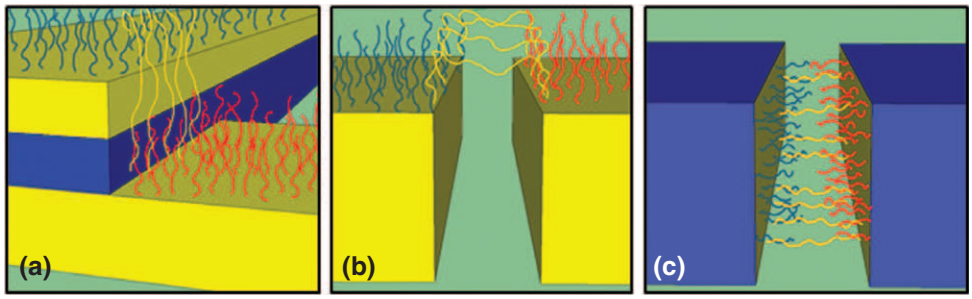

FIGURE 7 | Schematic representation of three platforms based on DNA wire sensors: (a) vertical,[54] (b) planar,[53] and (c) faced.[55] The red and blue oligonucleotides represent the two capture probes used in the detection of the DNA target (in orange).

observations about the sensitivity of DNA-mediated CT to π-stack structure alterations induced by mutation, prove the effectiveness of the sensing mechanism and the usability of this strategy for SNP genotyping. However, this platform does not have the low detection limits of the metallized wire DNA sensors.

CONCLUSION

The field of DNA biosensors has witnessed enormous growth, and consequently, great achievements have been established in recent years not only in developing applied detection technics but also in gaining a better picture of DNA physico-chemical properties. The lucky marriage between electrochemical analytical methods and the emerging nanotechnologies and nanomaterials shows promise for future development of sensor devices fulfilling the requirements imposed on clinical assays where detection of DNA sequences is involved. However, further improvements in reproducibility are mandatory to allow the development of commercially viable devices for wide-scale genetic screening. Efforts are now being devoted from one side to boost the current probe technology to avoid polymerase chain reaction (PCR) or similar target amplification strategies that are not ideal for point-of-care applications. This still represents a major challenge in the area of DNA detection. On the other side, a deeper understanding of DNA's physico-chemical properties and of the mechanisms of DNA-mediated CT, and better control of molecular interfaces could increase the hybridization efficiency and consequently the detection reproducibility. The recruitment of nanoscaled probes in the development of systems for the detection of DNA has already provided promising results. Despite this, a main drawback is still present. The nanotechnology required for nanosensor assessment and fabrication is costly and not fully reproducible. Reliable, low-cost fabrication methods need to be developed for competing with commercial devices, principally with DNA microarray platforms. The excellent progress in DNA analysis registered in the last 10 years, as shown by the corresponding dramatic reduction in the cost and time of analysis per genome, is a good omen for the future of this area.

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