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Electrochemical biosensor based on functional composite nanofibers for detection of K-ras gene via multiple signal amplification strategy

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ABSTRACT

An electrochemical biosensor based on functional composite nanofibers for hybridization detection of specific K-ras gene that is highly associated with colorectal cancer via multiple signal amplification strategy has been developed. The carboxylated multiwalled carbon nanotubes (MWCNTs) doped nylon 6 (PA6) composite nanofibers (MWCNTs–PA6) was prepared using electrospinning, which served as the nanosized backbone for thionine (TH) electropolymerization. The functional composite nanofibers [MWCNTs–PA6–PTH, where PTH is poly(thionine)] used as supporting scaffolds for single-stranded DNA1 (ssDNA1) immobilization can dramatically increase the amount of DNA attachment and the hybridization sensitivity. Through the hybridization reaction, a sandwich format of ssDNA1/K-ras gene/gold nanoparticle-labeled ssDNA2 (AuNPs–ssDNA2) was fabricated, and the AuNPs offered excellent electrochemical signal transduction. The signal amplification was further implemented by forming network-like thiocyanuric acid/gold nanoparticles (TA/AuNPs). A significant sensitivity enhancement was obtained; the detection limit was down to 30 fM, and the discriminations were up to 54.3 and 51.9% between the K-ras gene and the one-base mismatched sequences including G/C and A/T mismatched bases, respectively. The amenability of this method to the analyses of K-ras gene from the SW480 colorectal cancer cell lysates was demonstrated. The results are basically consistent with those of the K-ras Kit (HRM: high-resolution melt). The method holds promise for the diagnosis and management of cancer.

The ras gene, a proto oncogene, contains four exon sequences, distributes in the total length of approximately 30 kb DNA, and encodes a (mostly) nuclear phosphoprotein of 21 kDa [1,2]. H-, N-, and K-ras are the main members of the ras family. Ras family members normally cycle between active GTP-bound and inactive GDP-bound states, and they function at cellular membranes to transmit signals originating from extracellular stimuli to influence cell growth, proliferation, differentiation, and survival [3]. Oncogenic missense mutations at codons 12, 13, 61, and 146 strongly attenuate GTPase activity and cause ras to accumulate in the GTP-bound state, resulting in formation and progression of the cancer [4]. K-ras is the most frequently mutated ras isoform, with an incidence of approximately 15% across all human tumor types [5,6]. K-ras mutations have also been associated with increased tumorigenicity and poor prognosis [7,8]. Therefore, specific recognition and quantitative detection of K-ras and the mutations in K-ras are extremely crucial in fundamental research and clinical practice.

Up to now, a variety of methods for measuring K-ras mutation have been reported. The conventional electrophoresis-based methods, such as restriction fragment length polymorphism [9,10], denaturing gradient gel electrophoresis [11], and single-strand conformation polymorphism [12], are complicated and time-consuming. Furthermore, hazardous materials, including radioactive isotopes and ethidium bromide, were used. Direct sequencing [13,14] is expensive and not sensitive enough to detect low-abundant mutations. Real-time quantitative polymerase chain reaction (RQ–PCR)[1] [15,16] has the advantage of higher analytical

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[1] *Abbreviations used:* RQ–PCR, real-time quantitative polymerase chain reaction; MWCNT, multiwalled carbon nanotube; PA6, nylon 6; Py, pyrrole; ssDNA, single-stranded DNA; wtp53, wild-type p53; mtp53, muted-type p53; TH, thionine; PTH, poly(thionine); AuNP, gold nanoparticle; TA, thiocyanuric acid; FESEM. field-emission scanning electron microscopy; DPV, differential pulse voltammetry; CV, cyclic voltammetry; GC, glassy carbon; PBS, phosphate-buffered saline; dsDNA, double-stranded DNA; 3D, three-dimensional.

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sensitivity for mutation detection. However, the use of organic dye-labeled oligonucleotide probes makes its cost high. Compared with direct sequencing and RQ–PCR methods, SNaPshot assay [17,18] can simultaneously screen several mutations and reduce the cost of assays. Nevertheless, this method still requires expensive instruments and technical expertise. Recently, the DNA-based biosensor for the detection of K-ras mutations has generated considerable interest for simple, rapid, and inexpensive testing of genetic and infectious diseases. The sensors can translate the genetic recognition event into a corresponding analytical signal through electrochemical [19–21], optical [22–24], and electrochemiluminescence [25,26] methods. Among them, electrochemical DNA biosensors rely on the immobilization of a single-stranded oligonucleotide probe onto an electrochemical transducer surface to recognize its complementary target sequence. The high sensitivity of such devices, coupled with their high compatibility, portability, low cost, and minimal power requirements, makes them excellent candidates for DNA diagnostics [19–21].

Various nanomaterials have been used as electrochemical transducer surface to immobilize the oligonucleotide probe in electrochemical DNA biosensors during the past decade [27]. It is still a big challenge to make the biological recognition molecule utmost and stability immobilizing on the supporting scaffolds [28]. The nanofibers produced by electrospinning processes have advantages of uniformity, porosity, mechanical strength, and large surface areas [29,30]. It is believed that electrospun nanofibers can be used as an ideal platform for highly sensitive biosensing applications with the characteristics of high surface area for loading, capability of functional immobilization with desired spacing, reproducibility, and long-term storage [31]. Several reports have investigated the development of nanofiber-based biosensing platforms for biomolecular detection such as glucose [32] and protein [33]. But a biosensor based on electrospun nanofibers has never been studied to detect the ras gene.

Recently, we developed a novel electrochemical biosensor based on functional composite nanofibers for sensitive hybridization detection of p53 tumor suppressor using methylene blue as an electrochemical indicator [34]. In the strategy, carboxylated multiwalled carbon nanotubes (MWCNTs) doped nylon 6 (PA6) composite nanofibers (MWCNTs–PA6) were prepared using electrospinning, which served as the nanosized backbone for pyrrole (Py) electropolymerization. The functional composite nanofibers (MWCNTs–PA6–PPy) used as supporting scaffolds for single-stranded DNA (ssDNA) immobilization can increase the amount of DNA attachment. The biosensor displays good sensitivity, good specificity, and a high degree of discrimination between the wild-type p53 sequence (wtp53) and the muted-type p53 sequence (mtp53). To improve the detection sensitivity significantly, remarkable adjustments have been made to the sensing system. Here, an electrochemical biosensor based on functional composite nanofibers for hybridization detection of specific K-ras gene, which is highly associated with colorectal cancer via the multiple signal amplification strategy, is developed. The electrospun nanofibers (MWCNTs–PA6) served as the nanosized backbone for thionine (TH) electropolymerization via two steps (see Materials and Methods). The functional composite nanofibers [MWCNTs–PA6–PTH, where PTH is poly(thionine)], with larger specific surface area and good biocompatibility used as supporting scaffolds for ssDNA1 immobilization, not only can dramatically increase the amount of DNA attachment and the hybridization sensitivity but also can have better stability. Through the hybridization reaction, a sandwich format of ssDNA1/K-ras gene/gold nanoparticle-labeled ssDNA2 (AuNPs–ssDNA2) was fabricated. The AuNPs–ssDNA2 offered electrochemical signal transduction. An amplification strategy through the formation of the network-like thiocyanuric acid/gold nanoparticles (TA/AuNPs) was presented, and the detection sensitivity improved noticeably. Finally, the detection limit of the K-ras gene biosensor and the discrimination between the K-ras gene and the one-base mismatched sequence were improved to some extent compared with the p53 biosensor. The experimental protocol is illustrated in Fig. 1. In this study, the characteristics of the sensing system for the detection of K-ras gene mutations and the analytical performance were examined. The amenability of this method to the analyses of K-ras from the SW480 colorectal cancer cell lysates was demonstrated.

Materials and methods

Reagents and apparatus

The DNA oligonucleotides in this study were obtained from Sangon Biotechnology (China). The oligonucleotide sequences are

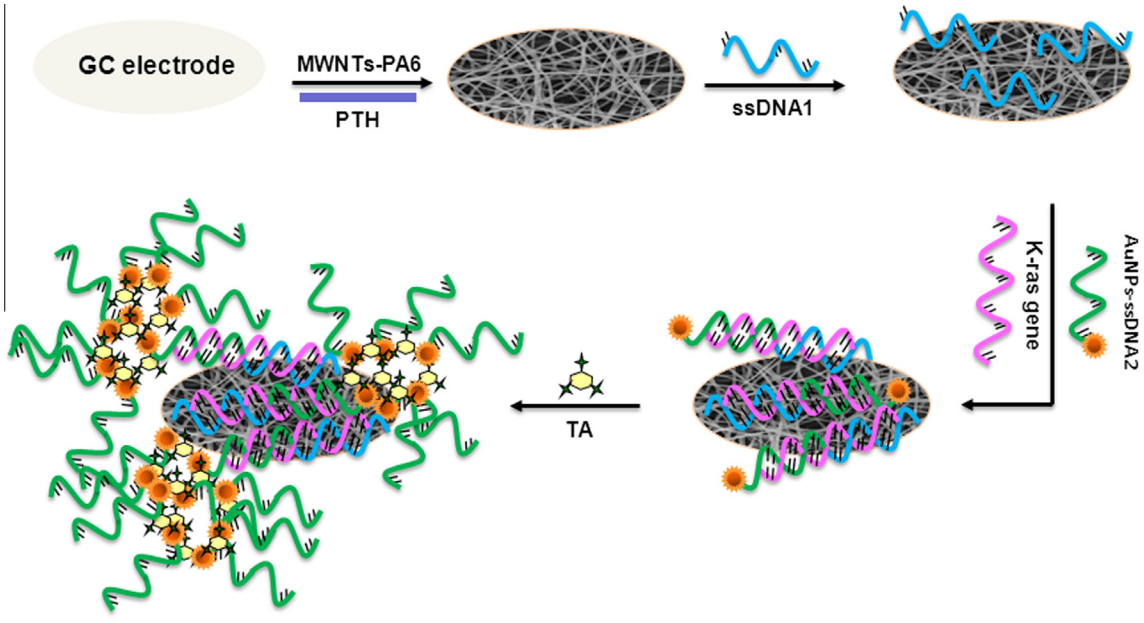

Fig.1. Schematic representation of the preparation of the electrochemical biosensor.