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A novel strategy for highly efficient isolation and analysis of circulating tumor-specific cell-free DNA from lung cancer patients using a reusable conducting polymer nanostructure

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ABSTRACT

We have developed a reusable nanostructured polypyrrole nanochip and demonstrated its use in the electric field-mediated recovery of circulating cell-free DNA (cfDNA) from the plasma of lung cancer patients. Although cfDNA has been recognized and widely studied as a versatile and promising biomarker for the diagnosis and prognosis of cancers, the lack of efficient strategies to directly isolate cfDNA from the plasma has become a great hindrance to its potential clinical use. As a proof-of-concept study, we demonstrated a technique for the rapid and efficient isolation of cfDNA with high yield and purity. In particular, the synergistic effects of the electro-activity and the nanostructured features of the polypyrrole polymer enabled repeated retrieval of cfDNA using a single platform. Moreover, polypyrrole nanochip facilitated the amplification of tumor-specific DNA fragments from the plasma samples of patients with lung cancer characterized by mutations in exons 21 of the epidermal growth factor receptor gene (*EGFR*). Overall, the proposed polypyrrole nanochip has enormous potential for industrial and clinical applications with significantly enhanced efficiency in the recovery of tumor-associated circulating cfDNA. This may ultimately contribute to more robust and reliable evaluation of gene mutations in peripheral blood.

1. Introduction

The exploration and analysis of circulating cancer biomarkers (i.e., circulating tumor cells (CTCs) and circulating cell-free DNA (cfDNA)) is of great significance for the early detection and screening of cancer [1–4]. Circulating cancer biomarkers are also important for establishing therapeutic strategies by tracing the molecular events that are closely implicated with cancer development, progression, and metastasis. In particular, tumor-related cfDNA, which possesses the hallmark characteristics of cancerous lesions, allows the discovery of detailed and comprehensive information on the nature of tumors through repetitive and serial monitoring [5,6]. In the new age of personalized medicine, therapies are directly tailored to the unique molecular and genetic features of tumors. Intensive efforts have been directed towards establishing minimally invasive and individually customized treatment strategies using circulating biomarkers, especially cfDNA, that are capable of detecting tumors and identifying genetic alterations [7–9]. It is well known that: i) primary and metastatic tumors readily shed small fragmented cfDNA into the bloodstream, probably as a result of tumor cell apoptosis and/or necrosis; ii) the levels of cfDNA in cancer patients are significantly higher in comparison with healthy individuals; and iii) elevated levels of circulating cfDNA in cancer patients are primarily associated with tumor burden, simultaneously reflecting a variety of genetic disorders [10,11]. Indeed, cfDNA has great potential to be more widely used as a reliable real-time tool for monitoring clinically relevant cancer-

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related genetic and epigenetic alterations. However, despite the importance of cfDNA, its clinical utility remains a challenge because current techniques lack sensitivity and specificity in the extraction of tumor-specific cfDNA from plasma, even with clinical samples of large volume (>1 mL) containing a reasonable concentration of target DNA [12–17]. Moreover, conventional methods are particularly suitable for direct use in the isolation and purification of high-molecular genomic DNA from cells or tissues [18,19]. Given the extreme rarity and highly fragmented nature (<200 bp) of tumor-specific cfDNA in plasma, the development of a new technique that is specifically designed to capture short fragments of DNA with a flexible sample volume range is urgently required for further clinical applications. We took advantage of the reversible changes in the electrochemical reduction-oxidation (redox) state of polypyrrole (Ppy) to develop a simple but highly efficient strategy for isolating and analyzing tumor-specific cfDNA using a reusable Ppy nanostructure (Ppy nanochip), as shown in Fig. 1. We have recently demonstrated an electroactive Ppy platform for the efficient capture and release of CTCs by electrically modifying the surface with desired dopant species [20–22]. Indeed, the electrochemical behavior and reversible redox properties of Ppy allow a more flexible transition of electrical charges along the polymeric backbones. By precisely manipulating intrinsic electrical activity, we can control the surface charges of Ppy in two distinct states: i) the oxidized state, in which the polymeric backbone is positively charged; and ii) the reduced, electrically neutral state [23–25]. Along with the redox stability and electro-activity of Ppy, association–dissociation interactions between the Ppy nanochip and negatively charged DNA molecules have been successfully accomplished in a facile and reversible manner. The electric field-mediated Ppy nanochip has several advantages: i) the Ppy nanochip, which undergoes electric potential-induced charge transitions, ensures the rapid and simple isolation of highly fragmented DNA from the plasma with significantly improved extraction and purification efficiency; ii) electrode regeneration can be easily and rapidly achieved by applying a negative potential, which allows the Ppy nanochip to be reused several times and permits the testing of multiple patient samples with a single electrode; and iii) Ppy nanochips can be applied to a range of different sample sizes (100 μL–3 mL), unlike conventional techniques that require a large volume of blood plasma and an optimal concentration of DNA to produce satisfactory clinical outcomes. Moreover, by precisely controlling the electropolymerization conditions, Ppy can be deposited onto indium tin oxide (ITO)-coated glass to achieve diverse surface roughness on a nanoscale. To impart additional nanoscale features to the surface of the Ppy nanochip, we successfully demonstrated electric field-generated nanorough surfaces that can readily influence the extraction performance by offering more active sites for the adherence of DNA arising from the larger surface area. The integration of the nanoroughened Ppy along with the applied electric potentials ultimately anticipates a synergistic effect in the isolation of fractionated cfDNA from lung cancer patients' blood. This effect is closely associated with the accurate identification and analysis of significant tumor-associated genes. We envision that our Ppy nanochip will be useful for the manipulation, isolation, and rapid detection of tumor-specific cfDNA in the blood.

2. Materials and methods

2.1. Fabrication and characterization of the Ppy nanochip

Ppy was electrochemically deposited on an ITO surface by applying an aqueous mixture of 0.1 M pyrrole and 0.01 M poly(-sodium 4-styrenesulfonate) (PSS) using chronoamperometry (CA) at 0.8–1.8 V (vs. Ag/AgCl) for 5 min. All electrochemical experiments were performed using a potentiostat/galvanostat (Biologic SP-50) in three-electrode cells, where platinum wire, Ag/AgCl, and the ITO served as counter, reference, and working electrodes, respectively. Subsequently, the synthesized Ppy was rinsed twice with distilled water and incubated in Tris-HCl buffer (pH 7.5) for additional electrochemical overoxidation at 1.8 V (vs. Ag/AgCl) for 2 min. The surface roughness of the Ppy platform was investigated by atomic-force microscopy (AFM) (Park Systems, XE-Bio).

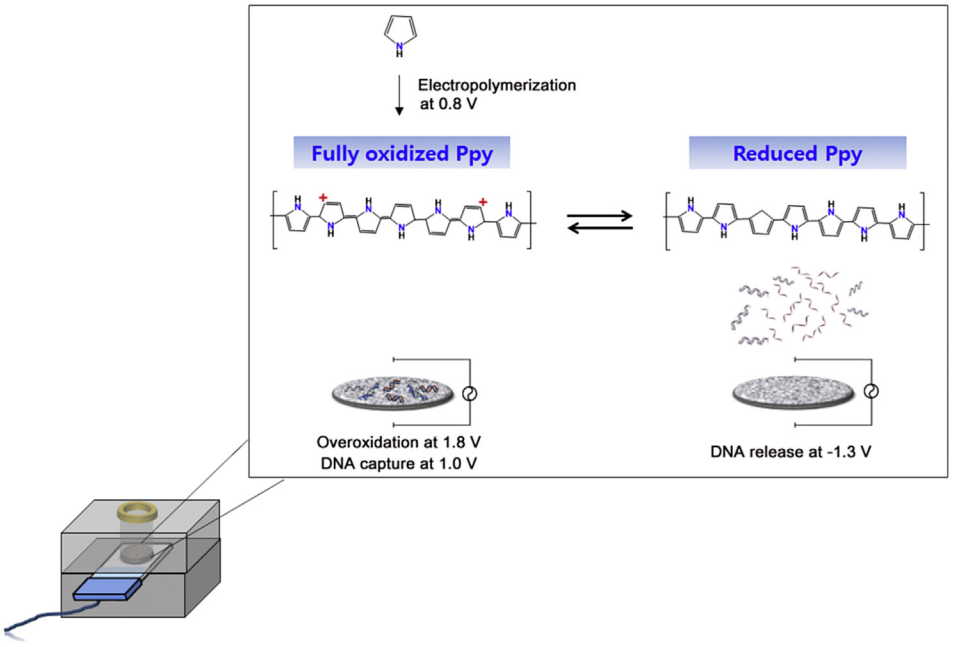

Fig. 1. Schematic illustration of the electric field-mediated recovery of circulating cell-free DNA (cfDNA) using the polypyrrole (Ppy) nanochip. The inset shows a reversible oxidation/reduction reaction of Ppy in response to the applied electrical potential that corresponds to the DNA recovery efficiency.