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Detection of circulating tumor cells in prostate cancer based on carboxylated graphene oxide modified light addressable potentiometric sensor

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ABSTRACT

Circulating tumor cells (CTCs) are a group of rare cancer cells that have detached from a primary tumor and circulate in the bloodstream. Herein, light addressable potentiometric sensor (LAPS) was exploited in the label-free detection of CTCs in the prostate cancer. To this end, the mouse anti-human epithelial cell adhesion molecule (anti-EpCAM) monoclonal antibody was selected as the probe to capture CTCs according to our western blot experiments, and therefore the anti-EpCAM was immobilized on the surface of carboxylated graphene oxide (GO-COOH) modified LAPS. Spiking experiments confirmed that LAPS' voltage decreased with the increasing of CTCs' concentration both in phosphate buffer (PBS) and blood, and as few as 10 CTCs in 1 ml of blood could be detected, illustrating the high sensitivity of the proposed strategy. The analysis of healthy blood samples revealed no change in electrical signal, confirming the specificity of the system. Ultraviolet–visible (UV–vis) spectroscopy, scanning electron microscopy (SEM), X-ray photoelectron spectroscopy (XPS) and immunofluorescent assay (IFA) were conducted to characterize GO-COOH, testify its existence on LAPS and validate CTCs' capturing by anti-EpCAM grafted on GO-COOH modified substrates. It is indicated that LAPS could be a potential platform for CTCs detection and may provide a powerful tool for downstream analysis.

1. Introduction

Circulating tumor cells (CTCs) are a group of rare cancer cells that have detached from a primary tumor and circulate in the bloodstream. The earliest discovery of CTCs dated back to 1869, when Ashworth reported a case in which tumor cells were found in the blood of a man with metastatic cancer. After observing microscopically CTCs in the peripheral blood, he postulated that "cells identical with those of the cancer itself being seen in the blood may tend to throw some light upon the mode of origin of multiple tumors existing in the same person" (Ashworth, 1869). Several subsequent studies have confirmed his finding. Moreover, numerous clinical trials have shown that high CTC numbers correlate with aggressive disease, increased metastasis, and decrease time to relapse (Chaffer and Weinberg, 2011; Plaks et al., 2013). Thus, CTC assay is regarded as a minimally invasive and real-time "liquid biopsy" for patients with cancer. CTCs have been used as a reliable marker to predict tumor response and patient survival, and they were detected even in the early stage of cancer (Cristofanilli et al., 2004; Goldkorn et al., 2014; Jiang et al., 2013).

Although CTCs could be used as a potential biomarker for clinical decision-making, their enrichment and isolation seems extremely important but difficult because of their rareness in the whole blood. CTCs are found in frequencies on the order of $1–10^3$/ml of peripheral blood in men with metastatic castration-resistant prostate cancer (Friedlander et al., 2014). However, the normal total leukocyte count ranges from 4×10^6 to 10×10^6/ml, and the normal erythrocyte count is up to 10^9/ml. Recently, there are several methods under development for CTC enumeration in cancer patients (Harouaka et al., 2014), whereas only one platform, the CellSearch system (Veridex, USA) is authorized by the FDA as a diagnostic tool for monitoring CTCs in the blood samples. It utilizes the anti-epithelial cell adhesion molecule (anti-EpCAM)

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magnetic beads to capture CTCs and follows with an additional cytokeratin, CD45, and DAPI staining to visualize the cells and differentiate from leukocytes (Bitting et al., 2013). However, the CellSearch assay is costly, time-consuming, and labor-intensive owing to multiple immunofluorescent staining. Hence, the development of novel methods for highly sensitive, label-free, and rapid detection is one of the most promising techniques for CTC analysis. Nanomaterials (e.g. nanoparticles, nanowires, and nanotubes) provide exciting new opportunities to improve CTCs detection efficiency (Lu et al., 2013; Wang et al., 2013). Graphene oxide (GO), a promising precursor of graphene, offers great potential for biological sensing because of its unique characteristics such as facile surface modification, good water dispersibility, and photoluminescence (Jung et al., 2010).

In this study, biofunctionalized light addressable potentiometric sensor (LAPS) is explored as a label-free platform of CTCs

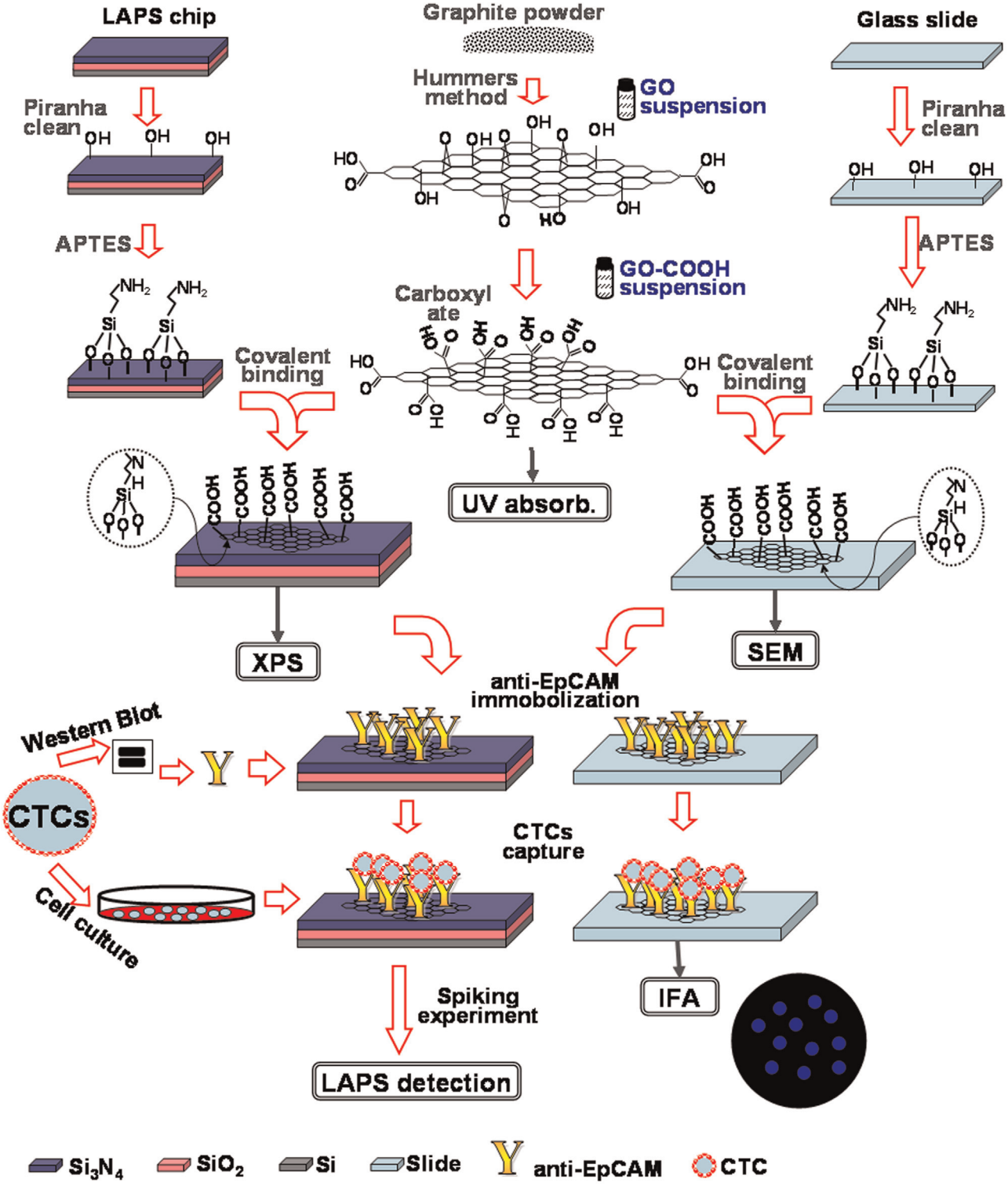

Fig. 1. A sketch map for the experiments of CTC-LAPS from material synthesis and characterization, chips' surface modification to cells' detection. CTC-LAPS experiments were in parallel with the immunofluorescent assay on the slides which were treated by the same procedure to validate the feasibility of the proposed strategy.