Journal of Pharmaceutical and Biomedical Analysis 117 (2016) 333–337

Contents lists available at ScienceDirect

Journal of Pharmaceutical and Biomedical Analysis

journal homepage: www.elsevier.com/locate/jpba

Short communication

A paper-based detection method of cancer cells using the photo-thermal effect of nanocomposite

Jianhua Zhou[a,1], Yanping Zheng[a,1], Jingjing Liu[a], Xin Bing[b], Jingjun Hua[b], Hongyan Zhang[a,*]

[a] College of Life Science, Shandong Normal University, Jinan 250014, PR China
[b] Shandong Product Quality Inspection Research Institute, Jinan 31000, PR China

ARTICLE INFO

Article history:
Received 3 April 2015
Received in revised form
14 September 2015
Accepted 15 September 2015
Available online 18 September 2015

Keywords:
Paper-based analytical device
Graphene nanocomposite
Photo-thermal effect
Cancer cell

ABSTRACT

A novel paper-based dot immune-graphene-gold filtration assay (DIGGFA) for the detection of breast cancer cells was developed based on the photo-thermal effect of graphene oxide (GO)-Au nanocomposite. Anti-EpCAM antibody which specific to the MCF-7 cell surface antigen, was immobilized on the nitrocellulose paper. The GO-Au-anti-EpCAM composite would interact with the MCF-7 cells captured on the nitrocellulose paper. After the test zone was irradiated by a laser, GO-Au nanocomposite could generate heat, temperature contrast was recorded and positive correlated with the cell number. Standard curve was prepared according to the temperature contrast and the cell number. Under optimal conditions, this method could detect a minimum of 600 MCF-7 cells with a near infrared laser and an infrared temperature gun within 15 min. This simple and rapid method could be applied to the clinical diagnosis in hospitals.

1. Introduction

Breast cancer is the most common female malignancy and a major cause of death in the world [1]. In order to diagnose breast cancer at an early stage, detection and identification of breast cancer cells is fundamental [1]. Currently, there are many different types of techniques to detect cells, such as immunocytochemistry [2], electrochemical methods [3], microfluidic devices [4] and biosensors [5]. Although they are very useful laboratory tests and have high sensitivity, many of these methods are expensive and require advanced instruments. Thus cheap, simple and rapid methods for detecting cancer cells need further studies, such as paper-based analytical devices (μPADs) [6] which can carry out detection, diagnosis and biochemical reaction on paper. μPADs include dot immunogold filtration assay [7] and lateral flow immunoassays [8]. In recent years, these simple paper-based methods have drawn much attention, but they were still less sensitive than other instrumental methods [9]. Research to improve the sensitivity of such paper based methods has become a primary focus. Wu et al. reported a lateral flow biosensor based on immunoassay for the detection of human stem cells for the first time [10]. It was capable of detecting a minimum of 10,000 human embryonic stem cells by visual judgment and 7000 cells with a portable paper strip reader within 20 min. Liu et al. reported an aptamer-nanoparticle paper strip biosensor for rapid, specific, sensitive, and low-cost detection of cancer cells [11]. This method was capable of detecting a minimum of 4000 Ramos cells by visual judgment and 800 Ramos cells with a portable strip reader within 15 min. The sensitivity of these paper-based detection methods can still be enhanced. Qin et al. significantly improved analytical sensitivity of lateral flow immunoassays based on the photo-thermal energy conversion of gold nanoparticles (AuNPs) [9]. The AuNPs conjugated with the target analyte were irradiated with a laser. Upon optical stimulation, AuNPs could generate heat and cause temperature variation. There was positive correlation between the concentration of target analyte and the temperature contrast, thus the concentration of target analyte could be calculated according to the linear curve. Enhanced photo-thermal energy conversion by attaching gold nanoparticles on graphene oxide (GO) has been demonstrated in several reports [12,13], so we tried to apply the GO-Au nanocomposite instead of AuNPs to improve the sensitivity of μPADs creatively.

Therefore, a novel dot immunographene-gold filtration assay (DIGGFA) was developed for the detection of Michigan cancer foundation-7 (MCF-7) cells using the photo-thermal effect of GO-Au nanocomposite. The GO-Au nanocomposite was modified by

* Corresponding author.
E-mail address: shwzhhy@163.com (H. Zhang).
[1] Joint first authors; these two authors contributed equally to this work.

http://dx.doi.org/10.1016/j.jpba.2015.09.017
0731-7085/

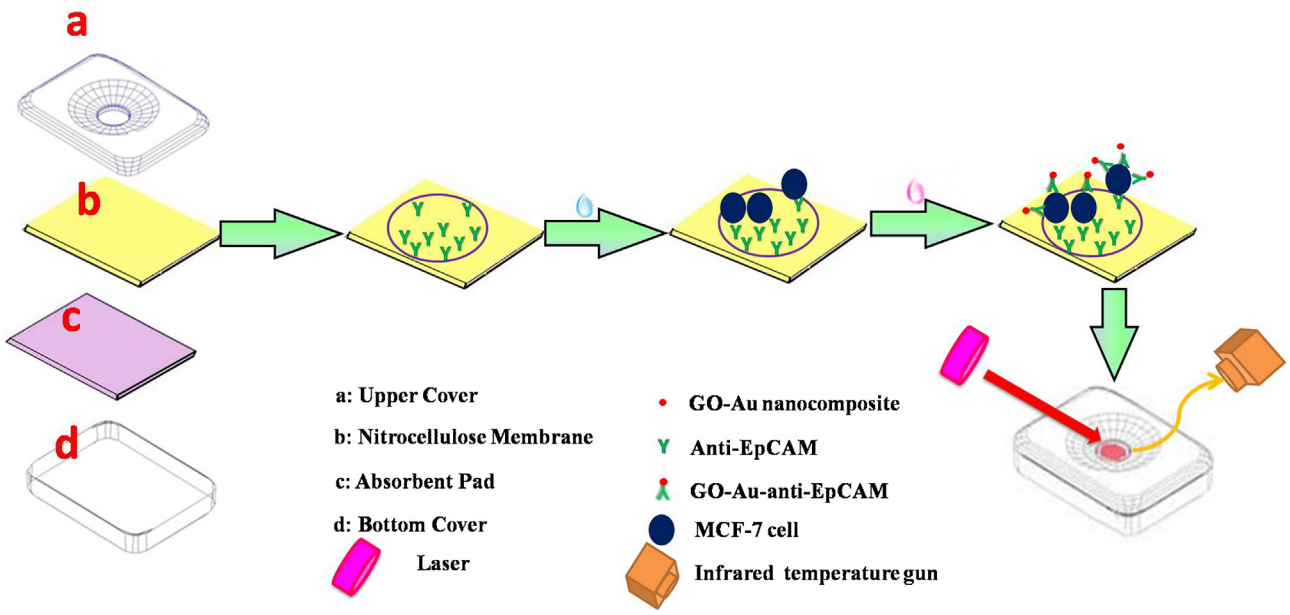

Fig. 1. The schematic diagram of DIGGFA.

the anti-epithelial cell adhesion molecule (EpCAM) antibody. So the recognition of cells to GO-Au nanocomposite was based on the specific binding of the EpCAM antibody with the antigen on the surface of the MCF-7 cells. The scheme of DIGGFA is illustrated in Fig. 1. Briefly, a nitrocellulose paper was coated with anti-EpCAM antibody which could capture the MCF-7 cells in the sample. The captured cells would interact with the anti-EpCAM antibody of the GO-Au-anti-EpCAM composite which was added subsequently. Thus, the GO-Au nanocomposite would be captured on the nitrocellulose paper. After the test zone was irradiated by a laser, GO-Au nanocomposite could generate heat, temperature contrast was recorded, and the number of cells was calculated. This simple, rapid, portable and low-cost method could fulfill the need of point-of-care testing and over-the-counter personal use.

2. Materials and methods

2.1. Chemical, materials and equipments

Anti-EpCAM antibodies were purchased from Bioss Biotechnology Co., Ltd., (Beijing, China). Bovine serum albumin (BSA) was purchased from Sigma–Aldrich (Steinheim, Germany). $HAuCl_4$ was purchased from Sinopharm Chemical Reagent Co., Ltd., (Shanghai, China). Potassium carbonate, trisodium citrate, methanol, Tween-20 and polyethylene glycol were from HuaTeHuaYan Science and Technology Co., Ltd., (Tianjin, China) and GO was from XFNANO Materials Technology Co., Ltd., (Nanjing, China).

MCF-7 cells were obtained from KeyGEN Biotechnology Co. Ltd., (Nanjing, China)

Nitrocellulose paper (Whatman AE99 and mid CLW-040) and absorbent pad were purchased from JieYi Bio-Technology Co., Ltd., (Shanghai, China). The digital heater (ZNHW) was purchased from Jiushi Science and Technology Co., Ltd (Shanghai, China). TEM images were taken by a Philips CM300 transmission electron microscope operating at an acceleration voltage of 100 kV. The near infrared laser OX-R301-1 was purchased from OXLASERS, the infrared temperature gun JXB-180 (the sensitivity is 0.1 °C) was purchased from Berrcom (Guangzhou, China).

2.2. Cell culture and collection

MCF-7 cells were maintained in Dulbecco's modified of Eagle's medium (DMEM) supplemented with 10% fetal bovine serumin, 5% CO_2, conditioned at 37 °C.

2.3. Preparation of GO-Au nanocomposite and conjugation with anti-EpCAM antibody

The procedure for the synthesis of the nanocomposite is described as follows. 1 mL 1% $HAuCl_4$ was diluted with double distilled water to 100 mL. The solution was transferred into a 100 mL round-bottom flask, heating until the water boiled. 2.5 mL sodium citrate solution was mixed under vigorous stirring [14]. The product was mixed with GO solution (2 mg/mL) when the color of the mixture changed into taupe, continue stirred until the color of the solution gradually changed into amaranth, kept stirring for another 10 min [15]. Stopped heating and cooled down the mixture to room temperature. Polyethylene glycol was added to modify the GO-Au [13], and the reduction reaction continued for another 1 h at an ultrasonic frequency of 211 kHz. Excess graphene oxide and polyethylene glycol were removed by centrifugation at 8000 rpm/min for 10 min. The sediment was redissolved in double distilled water, and the GO-Au nanocomposite was prepared.

A general procedure for the conjugation of GO-Au with anti-EpCAM antibody is described as follows. 1 mL GO-Au nanocomposite was adjusted to pH 9.0 with addition of 0.1 mol L^{-1} K_2CO_3, 1 mL 0.6 μg mL^{-1} anti-EpCAM antibody was added to the solution and the mixture was placed overnight at 4 °C. Finally, the resulting composite was centrifuged for 30 min at 10,000 rpm/min and redissolved in 200 μL stock solution.

2.4. Preparation of the μPADs

Nitrocellulose paper (pore size 8 μm, Whatman AE99) was fixed in a special plastic frame (3 cm × 3 cm × 1 cm) with a central well (diameter 0.8 cm). The paper was coated 1 μg/dot with anti-EpCAM antibody overnight. Blocking for 1 h at 37 °C, washed with modified phosphate-buffered saline (PBS) tween-20 (PBST) and then dried for storage at 4 °C until use. The absorbent pad (1.5 cm × 1.5 cm) was placed under the nitrocellulose paper in the plastic frame (Fig. 1).