Thin Solid Films xxx (2014) xxx–xxx

Contents lists available at ScienceDirect

Thin Solid Films

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

Joint detection of tumor markers with imaging ellipsometry biosensor

Yu Niu [a], Tengfei Kang [a,b], Gang Jin [a,*]

[a] NML, Institute of Mechanics, Chinese Academy of Sciences, 15 Bei-si-huan West Road, Beijing 100190, China
[b] University of Chinese Academy of Sciences, 19A Yu-quan Road, Beijing 100049, China

ARTICLE INFO

Available online xxxx

Keywords:
Joint detection
Tumor markers
Biosensor
Imaging ellipsometry

ABSTRACT

Tumor marker detection contributes to the early diagnosis of cancers. However, due to the lack of detection specificity, its results cannot act as a direct evidence to confirm cancer occurrence in clinic. Joint detection of tumor markers may improve the detection specificity. As a trial for clinical diagnosis of hepatocellular carcinoma, α-fetoprotein, α-L-fucosidase and ferritin have been combined and detected with a label-free, phase sensitive and high throughput imaging ellipsometry biosensor (IEB). Eighty-two sera have been quantitatively detected with IEB and the results are in agreement with the clinical standard approaches. Evaluated by receiver operating characteristic analysis, the specificity of joint detection improves remarkably with IEB for hepatocellular carcinoma. It can be foreseen that the joint detection of tumor markers with IEB has a potential for clinical cancer diagnosis.

1. Introduction

Cancer is the uncontrolled growth and spread of cells [1] and it has become a serious danger to human beings [2]. However, its survival chance is still basically dependent on tumor types, time of diagnosis and of course, clinical therapies [3]. The detection of tumor markers has been widely used to screen cancers on a population basis [4]. Although it contributes to cancer diagnosis and clinical therapies, tumor marker detection results cannot act as a direct evidence to confirm cancer presence because of the lack of detection specificity. In order to increase the detection specificity, several tumor markers are combined to detect cancers and improved results are acquired. However, joint detection of tumor markers is much more complicated than single marker detection and raises the requirement to detection methods. Thus, the need for effective methods to detect tumor marker combinations rapidly, sensitively and reliably is consequently subjected to broad interest.

Imaging ellipsometry [5,6] is derived from the conventional ellipsometry by introducing an expanded beam and a CCD camera instead of the traditional narrow beam and the photodiode detector. These changes render the unique advantages of conventional ellipsometry and microscope to imaging ellipsometry. Imaging ellipsometry is not only a label-free characterization technique with high resolution but it also owns a large field of view, providing the capability to detect a considerable area at the same time [7,8]. The concept of imaging ellipsometry biosensor (IEB) for visualization of biomolecular interactions was proposed in 1995 [9,10]. With the developments these years, it has become automatic equipment [11,12] for protein interaction analysis. IEB is primarily composed of a microfluidic reactor [13] and an imaging ellipsometer [10,14]. The microfluidic reactor is served to fabricate a patterned protein microarray; while the imaging ellipsometer is used to measure the surface mass concentration distribution of a protein microarray. So far, IEB has accumulated several application experiences in biological and clinical fields, for instance, five markers of hepatitis B virus [15], tumor markers [16,17], phage $M_{13}KO_7$ [18], severe acute respiratory syndrome virus [19], avian influenza virus [20] and ricin antibody identification [21].

Hepatocellular carcinoma is one of most common cancers in developing countries [2] and the average survival period is only about several months. Accurate diagnosis in an early stage has a potential to extend the survival period effectively. α-Fetoprotein (AFP) [22] and α-L-fucosidase (AFU) [23] are considered as two specific tumor markers for hepatocellular carcinoma; while ferritin [24] is commonly used to monitor lesion in liver tissue. In this investigation, the quantitative detection of these three tumor markers simultaneously has been performed with IEB as a trial.

2. Materials and methods

2.1. IEB and its detection principle

During the last decade, IEB has been updated and improved in our laboratory [11,12,14]. Now, it is mainly composed of a microfluidic reactor and an imaging ellipsometer.

The microfluidic reactor integrating 48 independent flow channels is used to fabricate protein microarrays by a series of continuous processes, including the surface patterning, the ligand immobilization, the surface blocking and the analyte solution delivery as well as the surface rinsing [12]. When a silicon wafer substrate is placed on the top of the reactor, it forms the 48 independent reaction cells and each cell has an independent inlet and outlet for the solution delivery. The inlet channels are connected to a sample reservoir and the outlet channels are connected with

* Corresponding author at: 15 Bei-si-huan West Road, Beijing 100190, China. Tel./fax: +86 10 82544138.
E-mail address: gajin@imech.ac.cn (G. Jin).

0040-6090/$ – see front matter © 2014 Elsevier B.V. All rights reserved.
http://dx.doi.org/10.1016/j.tsf.2014.01.043

the pumps offering negative pressure. With the microfluidic reactor, different solutions can be delivered to the appointed reaction cells and afterwards a microarray can be fabricated in a precise pattern.

The imaging ellipsometer acts as the data acquisition of a protein microarray. Imaging ellipsometry is an enhancement of the standard single beam ellipsometry, which combines the power of ellipsometry with microscopy and is worked in the off-null mode. It can be utilized for the visualization of surface mass concentration distribution of protein layers. A slight variation of surface mass concentration can be remarkably distinguished by the imaging ellipsometer and the result is represented in gray-scale images. Upon the high sensitivity requirements to observe biomolecule interactions, the imaging ellipsometer has been improved by the introduction of a spectroscopic light source and a low noise imaging device [12] with the optimization of polarization settings.

The principle to detect protein interactions with IEB is shown in Fig. 1 [11]. A ligand is immobilized on a surface to form a biosensing surface to a receptor which exists in an analyte solution. When the biosensing surface is exposed to the analyte solution, ligands and receptors can interact specifically with each other to form complexes due to their affinity. The surface mass concentration of protein layers will change during this recognition process. With the visualization of imaging ellipsometry, the change can be determined quantitatively in the format of gray-scale images. In that case, the existence of the receptor in the analyte solution can be verified.

2.2. Chemicals, samples and substrates

Silicon wafers are provided by Beijing GRINM Materials Company. 1-(3-Dimethylaminopropyl)-3-ethylcarbodiimide hydrochloride (EDC), succinic anhydride and aminopropyl-triethoxysilane (APTES) are purchased from Acros Organics. N-Hydroxy-succinimide (NHS), blocking buffer, phosphate buffered saline containing 0.05% Tween-20 (PBST) and protein A from *Staphylococcus aureus* are bought from Sigma-Aldrich. AFP and AFP rabbit monoclonal antibody are purchased from Sigma-Aldrich. AFU and its mouse monoclonal antibody are obtained from Abcam. Recombined ferritin and its goat monoclonal antibody are purchased from Sigma-Aldrich. 82 human serum samples including 41 healthy people and 41 hepatocellular carcinoma patients are collected in Shandong Academy of Medical Sciences. Their detailed clinical background and commercial immunoassay kit detection results are presented in Tables A.1 and A.2 in Appendix A. Deionized water is produced by ion exchange demineralization, followed by passing through a Milli-Q plus system from Millipore.

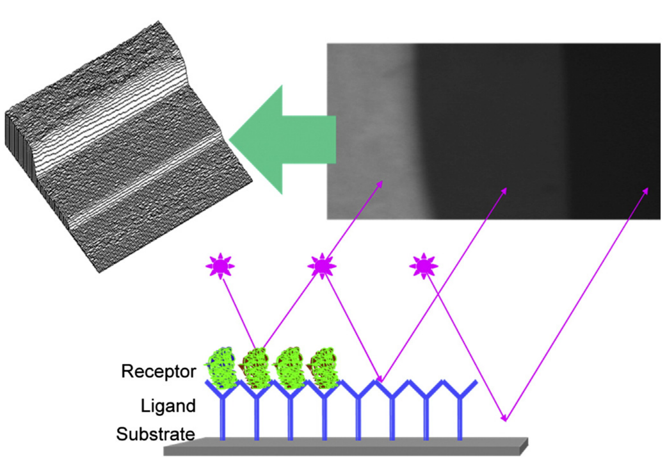

Fig. 1. IEB principle to detect protein interactions. The polarized light is incident on a protein layer and the reflective beam carries the information of the protein layer, for example its surface mass concentration. Ligand molecules can react with their receptors and form complexes on the biosensing interface, resulting in the change of layer surface mass concentration. This variation is embodied in reflection light intensity and can be quantitatively recorded in the format of gray-scale image [11].

2.3. Silicon wafer preparation and surface modification

Silicon wafer is used as a solid substrate for IEB. Due to the throughput need of 48 independent units, the silicon slides are cut into 25×13 mm^2 rectangular pieces. Then, in order to wash out the organic and inorganic contamination, silicon wafers are cleaned by a fresh piranha solution for 30 min. After being thoroughly rinsed with deionized water and pure ethanol, silicon wafers are incubated in an ethanol solution of APTES (5% APTES and 95% pure ethanol) for 2 h at room

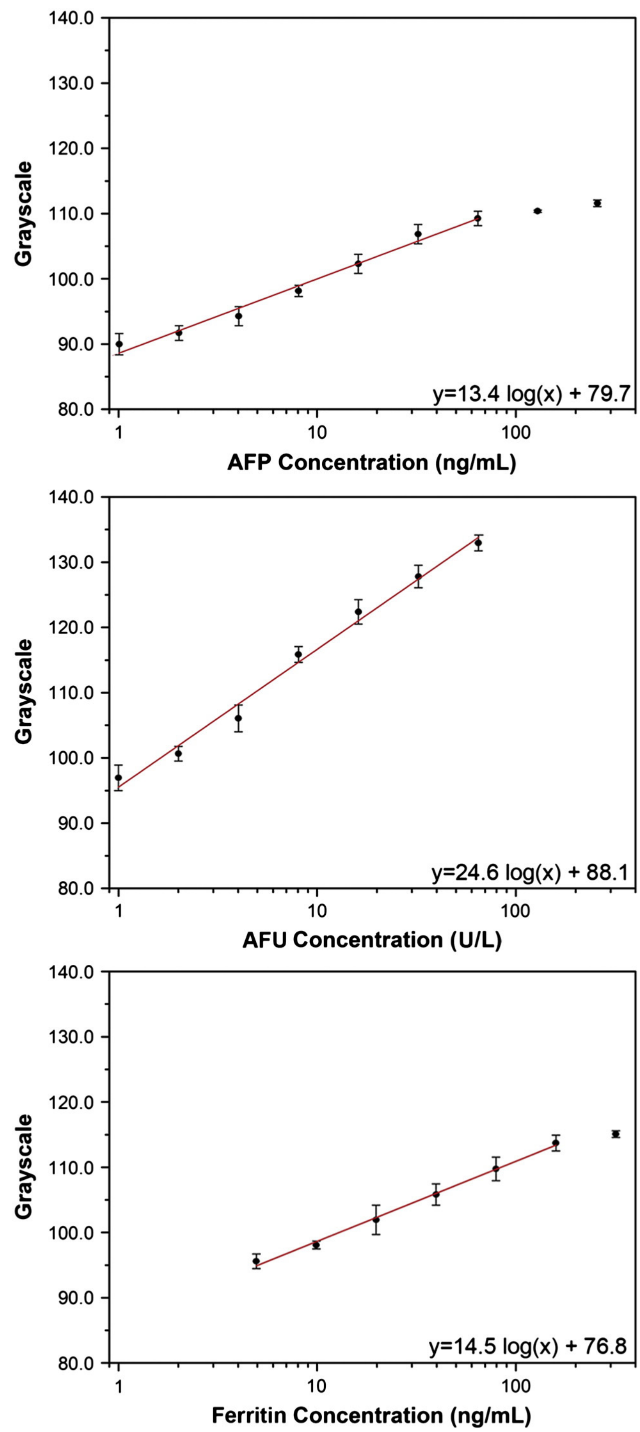

Fig. 2. The calibration curves for quantitative detection of AFP, AFU and ferritin. The calibration curves show that the relationship between IEB signals and the concentrations of AFP, AFU and ferritin is $y = 13.4\log(x) + 79.7$, $y = 24.6\log(x) + 88.1$, and $y = 14.5\log(x) + 76.8$, respectively, and the detection limit of AFP, AFU and ferritin is content to the clinical test standard.

Please cite this article as: Y. Niu, et al., Thin Solid Films (2014), http://dx.doi.org/10.1016/j.tsf.2014.01.043