Sensors and Actuators B 190 (2014) 165–170

Contents lists available at ScienceDirect

Sensors and Actuators B: Chemical

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

Procedure for developing linear and Bayesian classification models based on immunosensor measurements

Stephen Mobley[a,*], Sunil Yalamanchili[a], Hongzheng Zhang[b], Rossella Marullo[b], Zhuo G. Chen[b], Carlos S. Moreno[c], Dong M. Shin[b], Paul W. Doetsch[b], William D. Hunt[a]

[a] School of Electrical and Computer Engineering, Georgia Institute of Technology, Atlanta, GA 30332, USA
[b] Department of Hematology and Medical Oncology, Winship Cancer Institute of Emory University, Atlanta, GA 30322, USA
[c] Department of Pathology & Laboratory Medicine, Whitehead Research Building of Emory University, Atlanta, GA 30322, USA

ARTICLE INFO

Article history:
Received 15 May 2013
Received in revised form 23 August 2013
Accepted 28 August 2013
Available online 5 September 2013

Keywords:
Pattern recognition
Immunosensors
Cancer cell lysate
Head and neck cancer
Quartz crystal microbalance

ABSTRACT

A protocol for the creation of a set of classification models was developed to differentiate between biological samples based on immunosensor measurements. For this paper, data was gathered using Au Quartz Crystal Microbalance with Dissipation (QCM-D) sensors inoculated with an alkanethiol self-assembling monolayer functionalized for the detection of pAkt, γH2AX, β-Actin, and FITC antigen expression. Oropharyngeal cancer lysate samples, both positive (SCC47) and negative (TU212) for high risk human papillomavirus (HPV16), were used to gather the classification model training data set. Subsequently, linear and Bayesian classifiers were formulated based on the feature values and defined linear discriminant functions. The following study distinguishes between HPV-positive and HPV-negative cell lines, yet these guidelines can be utilized for different immunosensor platforms and disease diagnosis.

1. Introduction

Immunosensing is a field of study focused on the development of devices that detect target antigens based on the high affinity reactions to antibodies within a sensing platform. The antibody–antigen interaction was first exploited by immunoassay processes such as enzyme linked immunosorbent assay (ELISA). The primary goal of immunosensor development is to create simple point of care (PoC) or lab on a chip (LoC) systems [1].

Immunosensors are categorized into 4 groups based on their detection mechanism – electrochemical, optical, magnetic, or piezoelectric [1]. Piezoelectric sensors have been used to study the properties of a broad range of biochemical reactions. An emerging application of such sensors is the detection of biomarkers related diseases such as head and neck cancers.

Head and neck cancers (HNC) account for about 3% (34,540 cases) of new cancer cases per year in the United States and about 2% (7880 causalities) of all cancer deaths [2]. Common risk factors of cancers arising from oral cavity, larynx and pharynx are tobacco and alcohol abuse. As a consequence of decline in cigarette smoking habits, incidence rates of larynx and most oral cavity cancer sites is progressively decreasing [3]. In contrast with this tendency, the incidence rate of oropharyngeal carcinomas is increasing in the United States, indicating an emerging role for other carcinogens in this sub-group of HNC [3]. High risk human papillomavirus (HPV16) has been identified as the causative agent in oropharyngeal cancer pathogenesis [4], and it has been estimated that HPV-positive cancers account now for about 70–80% of all oropharyngeal tumors [5]. Recent clinical data suggest that HPV-positive status is an important prognostic factor associated with a favorable outcome in head and neck cancer patients treated with both radiation therapy and chemotherapy, alone or in combination [6,7].

Current biosensor platforms for the detection of HPV have focused on DNA as the target molecule. Electrochemical sensors have been developed for singular and multiplexed detection of high-risk strains of HPV [8,9]. Several piezoelectric platforms have also been developed such as a quartz crystal microbalance biosensor dependent on Polymerase Chain Reaction (PCR) to increase the concentration of target DNA [10]. Another example is the leaky surface acoustic wave (LSAW) device with the incorporation of bis-peptide nucleic acid (bis-PNA) for rapid detection of nominal concentrations of HPV DNA samples [11]. Detection of protein expression levels is more favorable for diagnosis of cancers than DNA due to the fact that irregularities in protein

* Corresponding author. Tel.: +1 404 245 5455.
E-mail addresses: smobley3@gatech.edu (S. Mobley), sunil8000@gmail.com (S. Yalamanchili), hzhan27@emory.edu (H. Zhang), rossella.marullo@emoryhealthcare.org (R. Marullo), gzchen@emory.edu (Z.G. Chen), cmoreno@emory.edu (C.S. Moreno), dmshin@emory.edu (D.M. Shin), medpwd@emory.edu (P.W. Doetsch), bill.hunt@ece.gatech.edu (W.D. Hunt).

0925-4005/$ – see front matter © 2013 Elsevier B.V. All rights reserved.
http://dx.doi.org/10.1016/j.snb.2013.08.081

concentrations alludes to issues within regulatory pathways in biological systems which is one of the causes of uncontrolled tumor growth.

In this study, the expression of four proteins in cell lysates, which are extract from cancer cells, were evaluated from HPV-positive (SCC47) and HPV-negative (Tu212) HNC cell lines. In particular, the expression of two proteins, γH2AX and pAkt, have been shown to correlate with cellular survival in response to radiation treatment [12,13]. Antibodies for FITC were used as a negative control to negate any non-specific binding. β-Actin antibodies were used as a positive control because they have a universal expression pattern that is conserved between both positive and negative cell types.

Quartz crystal microbalance (QCM) biosensors have been used as a means to detect the presence and properties of target molecules within both vapor and aqueous applications [14]. QCM sensors are bulk acoustic wave (BAW) cavity resonators consisting of a piezoelectric crystalline substrate. Depending on the thickness of the substrate, each device operates at a resonant frequency. Real-time tracking of the operational frequency serves as the detection mechanism for this technology.

Accuracy and efficiency are two critical components for immunosensors. This is typically addressed with the selection of surface chemistry to minimize factors such as non-specific binding. Yet, there are still many different factors that can alter results, such as sample preparation, sensor environment, and human error. A potential solution to these variables is the development of a classification system with techniques for normalizing the data. Previous sample differentiation has been constructed from linear regression models based on varying concentrations of the target molecule within purified solutions [15]. Pattern recognition statistical procedures allow for the creation of classification systems that show statistical significance in data discrimination.

Previous studies have used pattern recognition algorithms to analyze antibody–antigen interactions for different applications. Tsai et al. utilized multivariate statistical analysis of ELISA measurements to determine the influence of surface-bound fibrinogen on platelet adhesion to biomaterials [16]. Wagner et al. utilized principal component analysis to characterize absorbed protein films for biological implants based on time-of-flight secondary ion mass spectrometry measurements [17]. For these approaches, the developed models assess the target molecules properties based on post-procedure analytical methods. For this study, statistical information is extracted from raw immunosensor data which can give real-time concentration measurements; also defining different binding regions based on the dynamics of the antibody–antigen interaction.

In this study, we have utilized several pattern recognition algorithms to develop a class of differentiation models. For the linear classifier, Kozinec's algorithm was initially selected because it creates an optimal discriminant function that maximizes the marginal separation from the training data set [18]. One downside of linear classifiers is that they require the data set to be linearly separable or rather that the Gaussian regions that define the data set to have minimal intersection. Therefore, there is a need for a more complex model, which is achieved by a Bayesian classifier.

Bayesian classifiers utilize a priori probability to minimize the risk of a misclassification. The optimal solution to this problem then leads to the creation of the Bayesian divisor. This divisor defines regions of which subsequent trial values have a high probability to be assigned to the respective class [19]. Bayesian classifiers utilize probability density functions (PDF) in order to develop this classifier. Gaussian mixer models (GMMs), which parameters are calculated using the maximum-likelihood (ML) estimation, were selected to develop these PDFs.

The focus of this work was to develop a protocol for creating three types of classifiers ranging in complexity. This protocol was applied to a label-free QCM-D based immunosensor functionalized by an alkanethiol self-assembling monolayer (SAM) with binding affinities for pAkt, γH2AX, β-Actin, and FITC. Each model separated the training trials into two classes – HPV-positive (SCC47) and HPV-negative (TU212) cancer cell lines. The aforementioned technology and target proteins were selected as an example to illustrate the protocol's ability to define classification models.

2. Experimental

2.1. Reagents and materials

All antibodies were monoclonal IGg1 for β-Actin (Sigma–Aldrich), FITC (Santa Cruz), γH2AX (Cell Signaling), and pAkt (Cell Signaling). Reactants for the surface chemistry include 3,3′-dithiodipropionic acid (3,3″-DTP), 1-ethyl-3-(3-dimethylamino-propyl) carbodiimide (EDC) and N-Hydroxysuccinimide (NHS) were obtained from Sigma–Aldrich.

Squamous cell carcinoma of the head and neck (SCCHN) human cell line TU212 used for this study was established from a primary hypopharyngeal tumor. It was obtained from Dr. Gary L. Clayman's laboratory (The University of Texas M.D. Anderson Cancer Center, Houston, TX). The HPV-16-positive SCCHN cell line UM-SCC47 was obtained from Dr. Thomas Carey, University of Michigan. The tumor cell line was grown in DMEM/F12 (1:1) with supplemented 5% fetal bovine serum.

2.2. Antibody immobilization protocol

All Au-sensors were exposed to UV/Ozone treatment for 10 min, then submerged in a $H_2O/H_2O_2/NH_4$ (5:1:1) heated 75 °C for 5 min, and subsequently rinsed with DI water and exposed to UV/Ozone treatment for an additional 10 min.

Once housed in the QCM-D chambers, each sensor's resonant frequency was obtained using DI water as the initial surface medium. A self-assembled monolayer was then constructed on the surface of each sensor using 0.01 M 3,3′-DTP in ethanol for 10 min, and of 0.42 M/0.67 M EDC/NHS diluted in TAE buffer for an additional 10 min [20]. Each of the four sensors was then inoculated with a different antibody (β-Actin, FITC, γH2AX, and pAkt) at a concentration of 10 ng/ml diluted in PBS buffer until the frequency drift is less than 2 Hz/min.

2.3. Cell line preparation

Whole cell lysates were extracted using lysis buffer containing 50 mM Tris–Cl (pH 8.0), 150 mM NaCl, 0.02% Sodium azide, 0.2% SDS, 2% Igepal CA-630, 0.5% sodium deoxycholate and protease inhibitor cocktail (Sigma–Aldrich). Total protein content in the lysates was determined using the Bio-Rad protein assay (Bio-Rad).

2.4. QCM measurements

All trials were conducted in the flow chambers of the Q-Sense E4 module (Biolin Scientific/Q-Sense). The module records the frequency shift and dissipation of each chamber and displays the data in real-time. After antibody immobilization and a PBS buffer wash off, then a lysate sample (1:20 dilution in PBS) either SCC47 or TU212 was flown across all the sensors followed by a final PBS buffer wash off.