Clinical Biochemistry 45 (2012) 1689–1693

Contents lists available at SciVerse ScienceDirect

Clinical Biochemistry

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

Development of an Au/ZnO thin film surface plasmon resonance-based biosensor immunoassay for the detection of carbohydrate antigen 15-3 in human saliva

Yang-Hung Liang [a,b], Chia-Chen Chang [a], Chien-Cheng Chen [a], Yu Chu-Su [a], Chii-Wann Lin [a,*]

[a] Institute of Biomedical Engineering, National Taiwan University, Taipei 10617, Taiwan
[b] Tri-Service General Hospital, National Defense Medical Center, Taipei 11490, Taiwan

ARTICLE INFO

Article history:
Received 7 June 2012
Received in revised form 19 August 2012
Accepted 4 September 2012
Available online 12 September 2012

Keywords:
Saliva carbohydrate antigen 15-3
Surface plasmon resonance
Gold/zinc oxide
Biacore SPR

ABSTRACT

Objective: To evaluate a novel surface plasmon resonance (SPR) system for the direct measurement of tumor marker carbohydrate antigen 15-3 (CA15-3) in human saliva.

Design and methods: We measured the presence of the tumor marker carbohydrate antigen 15-3 (CA15-3) in human saliva using 2 different surface plasmon resonance (SPR) systems. To compare the sensitivity of an SPR biosensor based on thin-film Au/ZnO and the Biacore SPR system, we prepared CA15-3 samples in saliva and analyzed intensity responses to the samples at various concentrations of CA15-3.

Results: The linear detection range of CA15-3 in human saliva with the SPR system based on thin-film Au/ZnO was 2.5–20 U/mL (the cut-off point in cancer patients is around 4 U/mL). The linear range with the Biacore SPR system was 40–300 U/mL.

Conclusions: These results show that thin-film Au/ZnO-based SPR systems have higher sensitivity and can be used for measuring the levels of CA15-3 in human saliva without concentrating the samples.

Introduction

Saliva is an important bodily fluid and interest in it as a diagnostic medium has increased in recent years. Its main advantages are that it can be sampled non-invasively and can be collected repeatedly without the discomfort associated with taking blood samples. Saliva contains various substances and biomarkers that can be used as indicators of health and disease status. Successful measurement of salivary markers suggests that saliva samples may play diagnostic roles in many fields such as autoimmune disorders [1], cardiovascular diseases [2], infectious diseases [3], drug monitoring [4], cytokines [5], Alzheimer's disease [6] and cancers [7–9].

Carbohydrate antigen 15.3 (CA15-3), a 400-kDa glycoprotein of the MUC-1 mucin family, is present at higher levels in the serum and saliva of breast cancer patients than in healthy women. It is used as a reference marker, or "diagnostic gold standard," to which other breast cancer markers are compared [10]. Several studies have shown that the levels of CA15-3 in the saliva are beneficial in the diagnosis of early-stage breast cancer and in follow-up evaluations [11–13], but several difficulties for the routine use of this method still need to be overcome. First, the level of CA15-3 in saliva is up to 10-fold lower than that in serum [12]. Second, saliva is a complex material, and standardized collection and processing methods are essential for achieving reliable results [14,15]. Third, to our knowledge, there are no commercially available salivary CA15-3 standards or controls to evaluate the performance of this detection method. In this study, we have prepared varying concentrations of salivary CA15-3 samples and used different surface plasmon resonance (SPR) systems to detect CA15-3 at physiologically relevant concentrations.

SPR was introduced in the early 1990s as a novel, label-free biosensor technique [16]. SPR measures changes in the refractive index (RI) near a thin metal surface in response to molecular interactions, and the RI is directly correlated to the concentration of substances near the surface (in the medium). The detection system typically consists of a monochromatic, linearly polarized light source, a thin metal film in contact with the base of a prism, and a photodetector. SPR biosensors offer rapid screening of low concentrations of analytes in real time and detect biomolecules directly without labeling. These advantages make SPR assays a powerful quantitative tool in therapeutic drug monitoring (TDM) [17] and in the detection of biotoxins [18], herbicides and pesticides [19], cytokines [5], hormones [20,21], and tumor markers [22–24]. The Biacore SPR system is one of the most commonly used commercially available SPR sensors. This system provides accurate analysis through a completely integrated control system, a reusable sensor chip, and continuous flow technology that maintains a constant concentration of analyte in the bulk solution. However, assays of the low concentrations of CA15-3 found in saliva are challenging due to nonspecific binding and limitations of the sensitivity of the Biacore SPR system. In this study, we used a CM5 sensor chip in the Biacore SPR system. This chip comprises a glass slide with a thin layer of gold covered with a carboxymethylated dextran matrix to enhance the ligand immobilization capacity of the surface [25].

* Corresponding author. Fax: +886 2 23620586.
E-mail address: cwlinx@ntu.edu.tw (C.-W. Lin).

0009-9120/$ – see front matter
http://dx.doi.org/10.1016/j.clinbiochem.2012.09.001

The detection of CA15-3 in saliva by the Biacore SPR system was compared with the detection using an Au/ZnO thin film SPR system. Previous work from our laboratory showed that an Au/ZnO thin film SPR system could detect CA15-3 with high sensitivity (LOD = 0.025 U/mL) in pleural fluid samples [22] and could serve as a good alternative to conventional SPR immunosensors for detecting tumor markers. Our current results indicate that the Au/ZnO thin-film SPR system has sufficient sensitivity to measure CA15-3 levels in human saliva without first concentrating the samples. This SPR system may be useful as an important detection system for clinical diagnoses based on human saliva samples.

Experimental section

Materials

All chemical reagents used were of analytical grade. CM5 sensor chips, 1-ethyl-3-(3-dimethylaminopropyl) carbodiimide-HCl (EDC-HCl), *N*-hydroxysuccinimide (NHS), ethanolamine-HCl (ETA-HCl), and ethylenediaminetetraacetic acid (EDTA) were obtained from Biacore (Sweden). Carboxymethyl dextran (CM dextran sodium salt) and bovine serum albumin (BSA) were purchased from Sigma; 10× phosphate buffered saline (PBS) solution (pH 7.4) was purchased from Gibco-Invitrogen. 8-Mercaptooctanoic acid ($C_8H_{16}O_2S$; 8-MOA) was provided by Prof. Adam Shih-Yuan Lee from the Department of Chemistry at Tamkang University and was dissolved in absolute ethanol (ECHO Chemical). Monoclonal antibodies against the human breast carcinoma-associated antigen were purchased from Fitzgerald (USA).

Human saliva CA15-3 preparation

Thirty healthy female subjects aged 22 to 45 years (median age, 3 ± 27 years) participated in this study, and the protocol was approved by the Institutional Review Board of Tri-Service General Hospital. Written informed consent was provided by the participants. All the participants were asked not to eat or drink at least an hour prior to providing saliva samples. The subjects began a collection session by rinsing their mouths thoroughly with warm deionized water and then resting quietly for 5 min. Saliva was collected at 10:30 in the morning, and each participant provided 5 mL of saliva by unstimulated drooling into a 15 mL nonpyrogenic plastic tube (Falcon). The collection time was recorded to calculate the saliva flow rate. The saliva samples were frozen at −40 °C before being subjected to further treatment. The sample of human serum CA15-3 was collected from a breast cancer patient, and was provided by the laboratory of the Nuclear Medicine Department at Tri-Service General Hospital. To prepare the saliva CA15-3 samples, we replaced the serum matrix with pooled saliva using Amicon Ultra-0.5 Centrifugal Filter Devices (Amicon Ultra, 50 K molecular weight cut-off) according to the manufacturer's instructions. To confirm the different concentrations of CA15-3 in the saliva, each sample (300 μL) was assayed for CA15-3 in triplicate, using a commercial serum immunoradiometric kit (ELSA CA15-3; Cisbio, France). The measured intra-assay coefficients of variation (CVs) for low and high controls were 4.8% and 6.0%, respectively. The limit of assay detection was 0.2 U/mL.

Saliva CA15-3 processing before SPR analysis

We compared the effects of processing saliva containing CA15-3 by (1) centrifugation (4000 *g* for 10 min at 4 °C) and (2) passage through a 0.22-μm membrane syringe filter (Millex-GP; Millipore, Ireland). The recovery of saliva CA15-3 from the two processing methods was 82.5 ± 0.85% and 74.3 ± 0.96% (N = 3), respectively. Therefore, the centrifugation method was chosen as the saliva CA15-3 processing method.

Instrumentation

A Biacore X100 instrument was obtained from Biacore (GE, Sweden). Concentration analysis, instrument operation, and data processing were performed with Biacore X100 control software version 1.1. The SPRImager was purchased from GWC Technologies (USA).

Immobilization conditions on a CM5 sensor chip

Mouse monoclonal anti-human CA15-3 (Ma695) antibodies were immobilized on a CM5 sensor chip via an amine coupling process. The system was equilibrated with PBS buffer (pH 7.4) containing 0.05% Triton X100. The CM5 sensor chip was activated with a fresh mixture of EDC and NHS (1:1 v/v) maintained at a flow rate of 10 μL/min for 7 min. The CM5 sensor chip surface has two flow cells for immobilization [25]. In this study, we choose flow cell 2 (Fc2) to immobilize the Anti-human CA15-3 antibodies, and flow cell 1 (Fc1) as a reference surface. Anti-human CA15-3 (50 μg/mL) antibodies were injected over the Fc2 chip surface at a flow rate of 10 μL/min for 7 min. The surface was blocked with 1.0 M ETA-HCl at a flow rate of 10 μL/min for 7 min and nonspecifically bound antibodies were washed away with five 30-s injections of glycin buffer (pH 1.7). The resonance angle or signal in Biacore SPR is expressed in resonance units (RU). A response of 1000 RU corresponds to a change in surface concentration on the sensor chip of approximately 1 ng/mm^2 for proteins [25,34]. The final immobilized ligand density was 12,500 RU, corresponding to 12.5 ng/mm^2 (Fig. 1).

Fabrication of Au/ZnO chip substrates

Standard glass microscope slides (SF-10, Schott Glass) were used as base substrates. To remove any contaminants, the glass substrates were subjected to ultrasonic cleaning in piranha solution (H_2SO_4:H_2O_2 = 3:1) for 10 min and then rinsed 3 times with deionized water in an ultrasonic bath. The slides were dried with nitrogen gas. The Au films were deposited by an electron beam evaporator at a vacuum level of approximately 3×10^{-6} Torr. ZnO film was grown on a glass substrate using a radiofrequency sputtering system set to 13.56 MHz. A working pressure of 3 mTorr was employed during the deposition and a mixture of Ar (40%) and O_2 (30%) was used as the working gas.

Surface functionalization of the Au/ZnO film

Prior to surface functionalization, the gold-coated glass slide was washed with a detergent and ethanol solution and then rinsed with Milli-Q water followed by sonication. The gold-coated slide was then immersed into an 8-MOA solution at room temperature for 20 min to form a self-assembled monolayer on the surface. The chemically immobilized surface was activated with 400 mM EDC and 100 mM

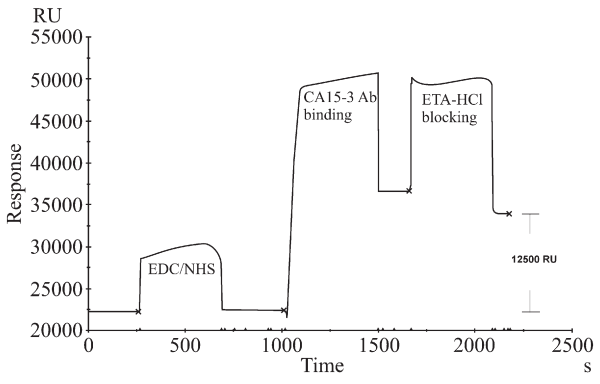

Fig. 1. Sensorgram of CA 15-3 antibody immobilized on a CM5 sensor chip. The final immobilized ligand density was 12,500 RU, corresponding to 12.5 ng/mm^2.

NHS for 10 min at room temperature. The gold-film chip was then soaked in a 50 μg/mL protein G solution that was allowed to fix onto the gold surface for 10 min. The anti-CA15-3 antibody, at a concentration of 50 μg/mL, was allowed to bind to the surface for 20 min, after which the surface was blocked with 1% BSA for 10 min. Binding reactions were monitored real time with the GWC SPRimager instrumentation as the analyte was passed over the sensor chip. The measured response values are expressed in arbitrary units (a.u.).

SPR data analysis

All experiments were run in triplicate. With data from the Biacore SPR system, analyses were performed with the Biacore X100 Plus Package evaluation software, version 1.1. With the Au/ZnO thin film SPR system, calibration curves were calculated using Sigma Plot software and analyzed with the following four-parameter logistic equation:

$$Y = B + \frac{(A-B)}{1+\left(\frac{X}{C}\right)^{D}},$$

where Y is the SPR intensity signal, X is the analyte concentration, A is the highest relative intensity of SPR detection, B is the lowest relative intensity of SPR detection, C is the concentration that exhibits the half maximal relative intensity, and D is the slope at the inflection point of the calibration curve.

Results and discussion

Human saliva CA15-3 preparation

Successful analysis of saliva requires consistent collection and processing methods [26,27] In this study, 30 healthy female subjects aged 22 to 45 years donated saliva by the same unstimulated spitting method at the same time of day and in the same location. The CA15-3 concentration in the saliva samples according to immunoradiometric measurement was 3.36 ± 0.36 U/mL, and the saliva flow rate was 0.37 ± 0.05 mL/min. These results are consistent with earlier measurements in a healthy control group [12]. In this study, we used pooled saliva to prepare the different concentration of saliva CA 15-3 calibrators to eliminate the different bulk refractive index response in the SPR measurement method caused by different saliva samples [25]. The serum matrix was replaced with pooled saliva upon passage through an Amicon Ultra-0.5 Centrifugal Filter Device. We compared 2 different filter sizes (50 K and 100 K molecular weight cutoffs) and found that the 50 K filter gave an 80% recovery rate as compared to only 30% for the 100 K device. We also used 2 saliva CA15-3 processing methods prior to analysis on the SPR systems and found that centrifugation yielded an 82.5 ± 0.85% recovery rate compared to a recovery of 74.3 ± 0.96% (N = 3) after passage through a 0.22-μm syringe filter. While we used centrifugation in this study due to its higher recovery rate, we recommend the filtration method for point-of-care testing due to its convenience.

Detection of saliva CA15-3 by the Biacore SPR system

The Biacore SPR system (GE, Sweden) is the most commonly used SPR sensor, and in this study, we used a Biacore X100 system with a CM5 chip for the detection of saliva CA15-3. The optimal equilibration solution was found to be PBS buffer (pH 7.4) mixed with 0.05% Triton X100, and the optimal regeneration solution was glycine buffer (pH 1.7). The baseline response was stable over 20 cycles, and we added carboxymethyl dextran (10 mg/mL) in the saliva CA15-3 solution to reduce non-specific binding [5,25]. Fig. 2 shows the sensorgrams of different CA15-3 concentrations in saliva, which were obtained using the Biacore SPR system.

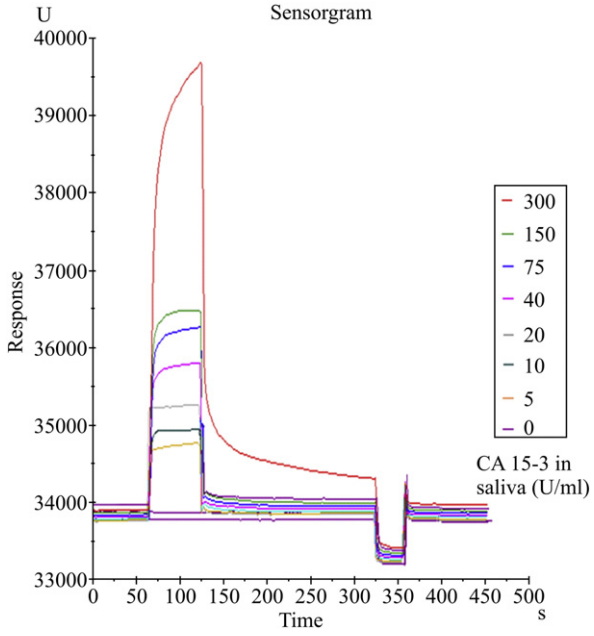

Fig. 2. Sensorgrams from the Biacore system for different saliva CA15-3 concentrations. *The 0 point of saliva CA15-3 is PBS buffer (pH 7.4) containing 0.05% Triton X100 solution.

We measured the dose–response relationship using several concentrations of saliva CA15-3 (0.10, 20, 40, 75, 150, and 300 U/mL, as confirmed by immunoradiometric measurements). The calibration curve (Fig. 3) was obtained from triplicate measurements of CA15-3 concentrations. The experimental data fit well to a four-parameter logistic equation [35], with a correlation coefficient (R^2) of 0.999.

Under our study conditions, no linear increase in intensity was seen with the Biacore X100 SPR system until the concentration of CA15-3 in the saliva reached 75 U/mL (inset in Fig. 3).

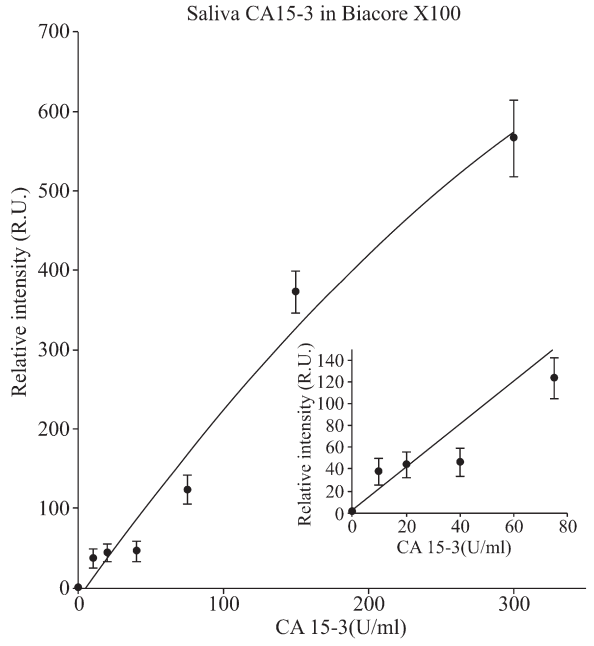

Fig. 3. Calibration curve for the detection of saliva CA15-3 (with solutions containing 0.10, 20, 40, 75, 150, and 300 U/ml) with the Biacore SPR system. The inset shows the response of the SPR biosensor from 10 U/mL to 75 U/mL with a correlation coefficient (R^2) of 0.868.

Detection of saliva CA15-3 by Au/ZnO thin film SPR

For comparison to the commercially available CM5 chip in the Biacore X100 system, we developed a Au/ZnO thin film chip using a radiofrequency sputtering system (13.56 MHz). The use of protein G to provide specific affinity-binding sites for interaction with antibodies [28,29] eliminated the random antibody couplings, resulting in high sensitivity and specificity. Our previous experimental report showed that the Au/ZnO thin-film SPR sensor had up to a 4-fold higher response than that of a conventional Au/Cr-based SPR sensor and could detect CA15-3 directly from pleural fluid samples [22]. The linear range for the determination of pleural fluid CA15-3 was from 1 U/mL to 40 U/mL and had good linearity, with an R^2 value of 0.991. This range is important for saliva CA15-3 detection because the level at which CA15-3 becomes a biomarker for cancer is around 4 U/mL [10]. Fig. 4 shows the SPR responses to the binding of 5 U/mL and 20 U/mL CA15-3 to the antibody-functionalized sensor surface. Compared to the Biacore X100 SPR system (Fig. 2), the Au/ZnO thin film SPR sensor gives better resolution at low concentrations of saliva CA15-3.

Fig. 5 shows the calibration curve obtained from triplicate measurements of saliva CA15-3 with the Au/ZnO thin film SPR sensor system. The data fit well to a four-parameter logistic equation with a correlation coefficient (R^2) of 0.987. The increase in intensity was linear from 2.5 U/mL to 20 U/mL CA15-3, with an R^2 value of 0.993 (inset in Fig. 5). The system became saturated at saliva CA15-3 concentrations over 20 U/mL, and such concentrations could not be quantified reliably. Previous reports from the Streckfus Lab [12] have shown that the saliva CA15-3 concentration for healthy controls is about 3.19 ± 0.52 U/mL; for benign breast tumors, about 7.23 ± 1.58 U/mL; and for malignant breast cancer tumors, about 10.90 ± 3.44 U/mL. The Au/ZnO thin film SPR sensor system used in this study showed a linear response to CA15-3 across this entire concentration range. Thus, this newly developed thin film SPR system is expected to be quite useful in the clinical diagnosis of cancer progression.

In this study, we found that the Biacore SPR system and Au/ZnO thin film SPR system had different detection ranges for saliva CA15-3. With the Biacore SPR system, the linear dextran in the CM5 chip provides an extensively solvated hydrogel that essentially behaves as a homogeneous layer that is approximately 100 nm thick [30,31]. This property might play a role in the detection of higher saliva CA15-3 concentrations; however, non-specific protein binding may decrease the sensitivity at lower concentrations. The low saliva CA15-3 concentration showed no linear response on the CM5 sensor chip in the Biacore SPR system. We assume that mass transfer effects might play an important role in this phenomenon [25,34]. In the CM5 sensor chip, the antibody is attached to a flexible non-crosslinked dextran polymer. CM5 matrix has intrinsic limitations in terms of steric hindrance, heterogeneities in the density, and conformation of probes inside the gel. The diffusion coefficient for CA15-3 is therefore reduced compared with the value in saliva solution. In addition, the viscosity in the surface layer is probably higher than in saliva solution, thereby reducing the diffusion coefficients for both the analyte and ligand. The dextran concentration in the surface matrix is close to 25 g/L, and at an immobilization level of 1000 RU, the protein concentration is approximately 10 g/L [25]. Diffusion-limited processes will therefore be slower than the corresponding processes in the saliva solution; furthermore, at low CA15-3 concentration, this effect might be more obvious. Non-specific protein binding might be another reason for decreased sensitivity at lower CA15-3 concentrations and might have resulted in the absence of linear response on the CM5 sensor chip [5]. In the future, we plan to design other systems and try the second available antibody that can recognize different epitopes on CA 15-3 surface to resolve this issue. It is also possible that thin-film Au/ZnO nanostructures might exhibit better optical and electrical properties than the gold-coated CM5 chip [31–33]. The K_D values for the Biacore and our Au/ZnO chip were calculated to be 381 and 17 U/mL, respectively. Obviously, the K_D of the Biacore system was much higher than that of our Au/ZnO SPR chip. This difference is mainly due to the limitations of the dextran matrix to present the probe in a homogeneous conformation with its target. CM5 matrix has intrinsic limitations in terms of steric hindrance, heterogeneities in the density and conformation of probes inside the gel, and kinetic resistance due to improper molecular orientation and mass transfer that cause an apparent decrease in the reaction-rate constant [36,37]. It would be interesting to continue a kinetic analysis in the future; currently, we are developing different systems that will allow further analysis of this issue and this will be a future goal of our research.

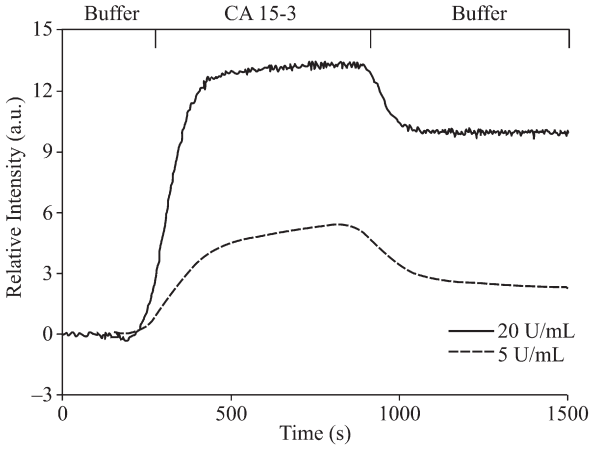

Fig. 4. Real-time intensity of saliva CA15-3 antigen as detected by the Au/ZnO thin film biosensor. The solid line represents the detection of the CA15-3 antigen at 20 U/mL. The dashed line represents the CA15-3 antigen (5 U/mL).

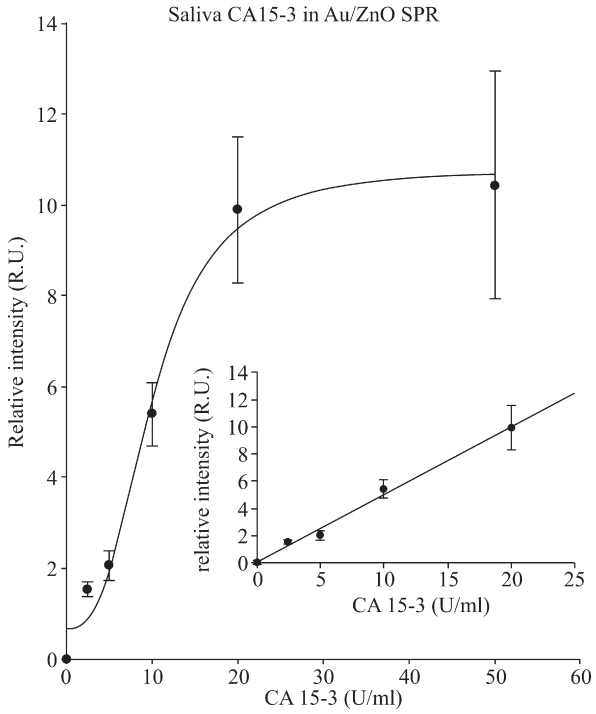

Fig. 5. Calibration curve for the detection of saliva CA15-3 (with solutions containing 0, 2.5, 5, 10, 20, and 50 U/mL) with an Au/ZnO-based biosensor. The inset shows the response of the SPR biosensor from 2.5 U/mL to 20 U/mL, with a correlation coefficient (R^2) of 0.993.

Conclusions

In this study, we compared the performance of a Au/ZnO thin film SPR system and a Biacore SPR system in the detection of CA15-3 in saliva. With the Biacore SPR system, higher concentrations of saliva CA15-3 could be detected, but the sensitivity of the system decreased as concentrations dropped below 75 U/mL. The Au/ZnO thin-film SPR system that we have developed showed a linear detection range below 20 U/mL, which covered all relevant CA15-3 concentrations from healthy individuals to those with malignant breast cancers. To our knowledge, this study is the first to compare different SPR biosensors for the measurement of CA15-3 concentrations in human saliva. We believe that the Au/ZnO thin film SPR system will prove useful as a diagnostic tool in detecting the progression of breast cancer.

Abbreviations

CA15-3	carbohydrate antigen 15-3
SPR	surface plasmon resonance
RI	refractive index
TDM	therapeutic drug monitoring
EDC-HCl	1-ethyl-3-(3-dimethylaminopropyl) carbodiimide-HCl
NHS	*N*-hydroxysuccinimide
ETA-HCl	ethanolamine-HCl
EDTA	ethylenediaminetetraacetic acid
BSA	bovine serum albumin
PBS	phosphate buffered saline
8-MOA	8-mercaptooctanoic acid
CV	coefficient of variation

Author contributions

The manuscript was written through contributions of all authors. All authors have given approval to the final version of the manuscript.

Funding sources

This work was supported by the National Science Council of the Republic of China under contract number NSC 95-2218-E-002-054-MY3.

Acknowledgments

We thank product specialist Sherry Lin of Biacore GE for the advice on this research.

References

[1] Talal N. Sjőgren's syndrome: historical overview and clinical spectrum of disease. Rheum Dis N Am 1992;18:507-15.
[2] Chatteron RT, Vogelsong KM, Lu YC, Ellman AB, Hudgens GA. Salivary alpha-amylase as a measure of endogenous adrenergic activity. Clin Physiol 1996;16:433-48.
[3] Tiwari SK, Khan AA, Ahmed KS. Rapid diagnosis of *Helicobacter pylori* infection in dyspeptic patients using salivary secretion: a non-invasive approach. Singapore Med J 2005;46:224-8.
[4] Drobitch RK, Svensson CK. Therapeutic drug monitoring in saliva. An update. Clin Pharmacokinet 1992;23:365-79.
[5] Yang CY, Brooks E, Li Y, Danny P, Montemagno CD. Detection of picomolar levels of interleukin-8 in human saliva by SPR. Lab Chip 2005;5:1017-23.
[6] Bermejo-Pareja F, Antequera D, Vargas T, Molina JA, Carro E. Saliva levels of Abeta1-42 as potential biomarker of Alzheimer's disease: a pilot study. Neurology 2010;10:1081-7.
[7] Boyle JO, Mao L, Brennan J. Gene mutations in saliva as molecular markers for head and neck squamous cell carcinomas. Am J Surg 1994;168:429-32.
[8] Warnakulasuriya S, Soussi T, Maher R, Johnson N, Tavassoli M. Expression of p53 in squamous cell carcinomas is associated with the presence of IgG and IgA p53 autoantibodies in sera and saliva of the patients. J Pathol 2002;192:52-7.
[9] Negri L, Pacchioni D, Calabrese F, Giacomasso S, Mastromatteo V. Serum and salivary CEA and GICA levels in oral cavity tumors. Int J Biol Markers 1988;3:107-12.
[10] Streckfus C, Bigler L. The use of soluble, salivary c-erbB-2 for the detection and post-operative follow-up of breast cancer in women: the results of a five-year translational research study. Adv Dent Res 2005;18:17-24.
[11] Hinestrosa MC, Dickersin K, Klein P, Mayer M, Noss K, Slamon D, et al. Shaping the future of biomarker research in breast cancer to ensure clinical relevance. Cancer 2007;7:309-15.
[12] Streckfus C, Bigler L, Dellinger T, Dai X, Kingman A, Thigpen JT. The presence of soluble c-erbB-2 in saliva and serum among women with breast carcinoma: a preliminary study. Clin Cancer Res 2000;6:2363-70.
[13] Agha-Hosseini F, Mirzaii-Dizgah I, Rahimi A. Correlation of serum and salivary CA15-3 levels in patients with breast cancer. Med Oral Patol Oral Cir Bucal 2009;14:e521-4.
[14] Groschl M, Kohler H, Topf H, Rupprecht T, Rauh M. Evaluation of saliva collection devices for the analysis of steroids, peptides and therapeutic drugs. J Pharm Biomed Anal 2008;47:478-86.
[15] Atkinson KR, Lo KR, Payne SR, Mitchell JS, Ingram JR. Rapid saliva processing techniques for near real-time analysis of salivary steroids and protein. J Clin Lab Anal 2008;22:395-402.
[16] Homola J. Present and future of surface plasmon resonance biosensors. J Anal Bioanal Chem 2003;377:528-39.
[17] Fu E, Chinowsky T, Miller JW, Yanger P. SPR imaging-base salivary diagnostics system for the detection of small molecule analytes. Ann N Y Acad Sci 2007;1098:335-44.
[18] Haughey SA, Campbell K, Yakes BJ, Prezioso SM, DeGrasse SL, Kawatsu K, et al. Comparison of biosensor platforms for surface plasmon resonance based detection of paralytic shellfish toxins. Talanta 2011;85:519-26.
[19] Rajan SC, Gupta BD. Surface plasmon resonance base fiber-optic sensor for the detection of pesticide. Sens Actuators, B 2007;123:661-6.
[20] Stevens RC, Soelberg SD, Near S, Furlong CE. Detection of cortisol in saliva with a flow-filtered, portable surface plasmon resonance biosensor system. Anal Chem 2008;80:6747-51.
[21] Miyashita M, Shimada T, Miyagawa H, Akamatsu M. Surface plasmon resonance-based immunoassay for 17ß-estradiol and its application to the measurement of estrogen receptor-binding activity. Anal Bioanal Chem 2005;381:667-73.
[22] Chang CC, Chiu NF, Lin DS, Chu-Su Y, Liang YH, Lin CW. High-sensitivity detection of carbohydrate antigen 15-3 using a gold/zinc oxide thin film surface plasmon resonance-based biosensor. Anal Chem 2010;82:1207-12.
[23] Chou SF, Hsu WL, Hwang JM, Chen CY. Development of an immunosensor for human ferritin, a nonspecific tumor marker, based on surface plasmon resonance. Biosens Bioelectron 2004;19:999–1005.
[24] Karlsson R. SPR for molecular interaction analysis: a review of emerging application areas. J Mol Recognit 2004;17:151-61.
[25] BIA Technology handbook, version AB, Sweden; 1998.
[26] Henson BT, Wang DT. Collection, storage, and processing of saliva samples for downstream molecular application. Oral Biol Methods Mol Biol 2010;666:21-30.
[27] Helton KL, Nelson KE, Fu E, Yanger P. Conditioning saliva for use in a microfluidic biosensor. Lab Chip 2008;8:1847-51.
[28] Babacan S, Pivarnik P, Letcher S, Rand AG. Evaluation of antibody immobilization for piezoelectric biosensor application. Biosens Bioelectron 2000;15:615-21.
[29] Muñoz E, Vidarte L, Pastor C, Casado M, Vivanco F. A small domain of bacterial protein G inhibits C3 covalent binding to the Fc region of IgG immune complexes. Eur J Immunol 1998;28:2591-7.
[30] BIAcore X100 handbook BR-1008-10 AB; 1998. Sweden.
[31] Liao H. Coupled whispering gallery modes in a multilayer-coated microsphere. Opt Lett 2003;28:1970.
[32] Wang X, Kong X, Yu Y, Zhang H. A highly efficient Ag–ZnO photocatalyst: synthesis, properties, and mechanism. J Phys Chem C 2007;111:3836-41.
[33] Mosbacker HL, Strzhemechny YM, White BD, Smith PE, Look DC, Reynolds DC, et al. Role of near-surface states in ohmic–Schottky conversion of Au contact to ZnO. J Appl Phys Lett 2005;87:12102-3.
[34] Stenberg E, Persson B, Roos H, Urbaniczky C. Quantitative determination of surface concentration of protein with surface plasmon resonance using radiolabeled proteins. J Colloid Interface Sci 1991;143:513.
[35] Findlay JWA, Dillard RF. Appropriate calibration curve fitting in ligand binding assays. AAPS J 2007;9:E260-7.
[36] Schuck P. Reliable determination of binding affinity and kinetics using surface plasmon resonance biosensors. Curr Opin Biotechnol 1997;8:498-502.
[37] Yarmush ML, Patankar DB, Yarmush DM. An analysis of transport resistances in the operation of BIAcore™; implications for kinetic studies of biospecific interactions. Mol Immunol 1996;33:1203-14.