



Sensitive and selective analysis of a wide concentration range of IGFBP7 using a surface plasmon resonance biosensor



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ABSTRACT

A sensitive method for selectively detecting insulin-like growth factor-binding protein 7 (IGFBP7) over a wide range of concentrations based on the surface plasmon resonance (SPR) biosensing techniques is described. IGFBP7 has been shown to regulate cell proliferation, cell adhesion, cellular senescence, apoptosis, and angiogenesis in several different cancer cell lines. Since the concentration of IGFBP7 can vary widely in the body, determining the precise concentration of IGFBP7 over a wide range of concentrations is important, since it serves as an inducible biomarker for both disease diagnosis and subsequent therapy. The SPR sensing method is based on the selective interaction of IGFBP7 with specific anti-IGFBP7 proteins on a gold thin film, which was covalently bound to the Fc-binding domain of protein G on a mixed self-assembled monolayer composed of DSNHS ($S_2(CH_2)_{11}COO(CH_2)_2COO-(N\text{-hydroxysuccinimide})$) and mercaptoundecanol, and effect of this on changes in the SPR profiles. The limit of detection (LOD) of the SPR biosensor was determined to be 10 ng/ml, which is a reasonable LOD value for biomedical applications. The response is essentially linear in the concentration range of 10–300 ng/ml. The SPR biosensor also shows specificity for IGFBP7 compared to that for biologically relevant interleukin (IL) derivatives including IL4, IL23, IL29, and IFG1. These molecules are also present along with IGFBP7 in the cell culture medium and have the potential to interfere with the analysis. Finally, the level secretion of IGFBP7 from cancer cells detected by the SPR biosensor showed a good correlation with a commercial kit using an IGFBP7 enzyme-linked immunosorbent assay. The findings reported herein indicate that the SPR biosensor for IGFBP7 would be applicable in a wide variety of biomedical fields.

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1. Introduction

The insulin-like growth factor-binding protein 7 (IGFBP7), also called IGFBP-rP1 or MAC25, is a member of the IGFBP superfamily, which includes 16 secreted proteins that bind IGFs with variable affinity [1]. IGFBP7 binds IGFs with a low affinity, but in contrast, has a high affinity for binding insulin, which modifies its metabolism, distribution and ability to bind to insulin receptors. A large body of evidence suggests that IGFBP7 regulates cell proliferation, cell adhesion, cellular senescence, apoptosis, and angiogenesis in

different cancer cell lines, and it is therefore considered to function as a tumor suppressor gene in numerous cancers [2–5]. Furthermore, it has recently been reported that IGFBP7 is associated with human acute kidney injury and lung cancer. Because of this, IGFBP7 has been studied and validated as an important biomarker. In more detail, IGFBP7 is known to be downregulated in human lung cancer due to the methylation of DNA [6–9]. In addition, IGFBP7 has been reported to be a novel prognostic urinary marker in early acute kidney injury [10–12]. In human bodies and in individual cells, IGFBP7 is present at various concentrations. For instance, when IGFBP7 is used as a biomarker, it is frequently detected at concentrations ranging from few to several tens of ng/ml [6–12]. On the other hand, in some reports of the tumor-suppressing effect of IGFBP7 for hepatocellular carcinomas, the determined concentration of IGFBP7 was in the range of several hundreds of ng/ml [13,14].

Most assays used to detect IGFBP families involve labeling, and include an enzyme-linked immunosorbent assay (ELISA) [15–17] and a fluorescence assay [18–20]. Among the conventional assays,

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ELISA is one of the most frequently used methods for the detection of cytokine levels in serum or cell culture media, since it possesses a high specificity and sensitivity. However, these label-based assays have critical drawbacks when used in the direct monitoring of IGFBP families over a wide range of concentrations. In these methods, a stock sample solution needs to be sequentially diluted to obtain the desired concentration range. This complicated procedure can cause the propagation of errors in dilution, leading to serious inaccuracies in an assay [21,22]. Moreover, the methods are time-consuming, requiring 6–8 h to complete an analysis, which makes it difficult to use in situations requiring real-time monitoring. Therefore, the development of a simple and direct method that can determine the precise concentration of IGFBP7 over a wide concentration range is clearly an important issue.

Here we describe a label-free sensitive and selective method for the quantification of IGFBP7 over a wide range of concentrations, based on a surface plasmon resonance (SPR) biosensing technique. The sensing method involves the selective binding of IGFBP7 to anti-IGFBP7 proteins and changes in the SPR signal caused by the interaction between IGFBP7 and anti-IGFBP7 proteins. SPR-based biosensors have attracted interest for detection of biomolecular interactions, are sufficiently sensitive to detect the small changes in the refractive index near the sensor substrate [23,24]. In particular, the high specificity of interaction between certain receptors and the analytes has been applied to the design of SPR biosensors, thus permitting highly selective detection to be achieved [25,26]. SPR biosensors are also useful for sensing the presence and amount of analytes, as well as for real-time monitoring by measuring the kinetics of association/dissociation at the interface [27,28].

In order to reduce non-specific binding and enhance the sensitivity with a wide range of detection, a specific anti-IGFBP7 protein was fabricated on a gold thin film, which was covalently bound to the Fc-binding domain of protein G on a mixed self-assembled monolayer (SAM). The proposed SPR biosensor for IGFBP7 thus has the potential for use in sensing trace amounts. The SPR biosensor can also selectively bind with a stronger affinity to IGFBP7 compared to biologically relevant molecules, such as interleukin (IL) derivatives including IL4, IL23, IL29, and IFG1, which can also be present along with IGFBP7 in cell culture medium and could interfere with the detection. Finally, we confirmed that our biosensor is feasible, by selectively detecting the molecule in a culture medium of cancer cells. We believe that the SPR biosensor for IGFBP7 would be applicable in many biomedical fields.

2. Experimental

2.1. Materials

Gold thin film (K-MAC), $S_2(CH_2)_{11}COO(CH_2)_2COO$ -(*N*-hydroxysuccinimide) (DSNHS, K-MAC Ltd.), phosphate buffered saline (PBS, Sigma–Aldrich), protein G (Sigma–Aldrich), mercaptoundecanol (MUO, Sigma–Aldrich), H_2O_2 (Sigma–Aldrich), H_2SO_4 (Sigma–Aldrich), ethanolamine hydrochloride (Sigma–Aldrich), anti-IGFBP7 (R&D SYSTEM Ltd.), IGFBP7 (R&D SYSTEM Ltd.), IFG1 (R&D SYSTEM Ltd.), IL4 (PEPROTECH Ltd.), IL23 (e-Bioscience Ltd.), IL29 (Cell Signaling Ltd.) were used as received. A piranha solution (H_2O_2 and H_2SO_4 , Sigma–Aldrich) was used to clean the gold thin film. All chemicals were used as received.

2.2. SPR measurements

A flow cell (50 μ l/min, 25 °C) was mounted on the sensor/prism assembly so that solution of interest could be introduced so as to easily flow across the surface of gold thin film substrate and that switching between different solutions could be accomplished

rapidly (SPR *micro*, K-mac Ltd). The SPR system was utilized in the Kretschmann configuration using attenuated total reflection (ATR). Time-resolved SPR angle shifts were measured using the fixed angle method which enabled the reflectance change, ΔR , to be linearly correlated with the SPR angle shift, $\Delta\theta_{SPR}$. Reflectance data at a fixed incident angle were acquired in real time on a computer [29,30].

2.3. Fabrication of SPR sensor surface

The gold thin film modified with MUO and DSHHS was prepared to immobilize the anti-IGFBP7 proteins on the substrates. A mixed monolayer (SAM) of MUO and DSNHS was self-assembled on the gold thin film by treating it overnight with a 4:1 mixture of a 4.0 mM chloroform solution at room temperature. Immobilization procedure was carried out using a SPR*micro* instrument. Protein G was diluted to a concentration of 100 μ g/ml with PBS and immobilized on the mixed SAM by means of amine-coupling chemistry. Excess activated carboxyl groups were blocked by treatment with 1.0 M ethanolamine. The anti-IGFBP7 solution of 10 μ g/ml was then injected onto the protein G-modified gold thin film.

2.4. Quantification of IGFBP7 by SPR

A standard solution of IGFBP7 was prepared by dissolving it in PBS. Standard IGFBP7 solutions, in a concentration range from 10 ng/ml to 1.0 μ g/ml, were injected into an inlet of the sample well on the modified gold thin film surface, and real-time signals of the interface of the gold thin film that were induced by the interaction between the receptors and IGFBP7 were then measured. Signal changes in reflectance were collected and the standard deviation for each concentration of IGFBP7 was determined.

2.5. Cell culture

Human lung carcinoma A-549 cells were grown in RPMI (Roswell Park Memorial Institute) 1640 supplemented with 10% fetal bovine serum, streptomycin of 100 μ g/ml and penicillin of 100 IU/ml. A confluent cell number was seeded in a plate, the media altered to FBS-free under 37 °C and 5% CO_2 for 2 days. The supernatant from the culture medium was collected at 48 h and reserved for further SPR and ELISA analysis.

2.6. ELISA measurement

A commercial ELISA was used to evaluate the accuracy of the IGFBP7 level determined by the SPR biosensor. The supernatant from the cultured cells was collected and centrifuged at 1500 rpm for 10 min. The concentration of IGFBP7 was determined using an ELISA kit for IGFBP7 (SEB973Hu, Science Inc.).

3. Results and discussion

3.1. Immobilization of the IGFBP7 antibody ligand

A schematic illustration for the method is shown in Fig. 1. In the antibody immobilization methods, anti-IGFBP7 proteins were indirectly immobilized to the Fc-binding domain of protein G on a gold thin film by covalent attachment to a mixed SAM surface via the formation of an amide bond. The mixed SAM was fabricated using DSNHS and MUO to reduce steric hindrance effects that control ligand density and effectively prevent the non-specific adsorption of receptors by inducing the partial formation of ω -carboxyalkylthiol groups on the substrate [31–35]. Moreover, since the reactive site of DSNHS is tethered to a molecular component of a mixed SAM that is longer than MUO comprising the surrounding organic background, this additionally reduces the steric effects

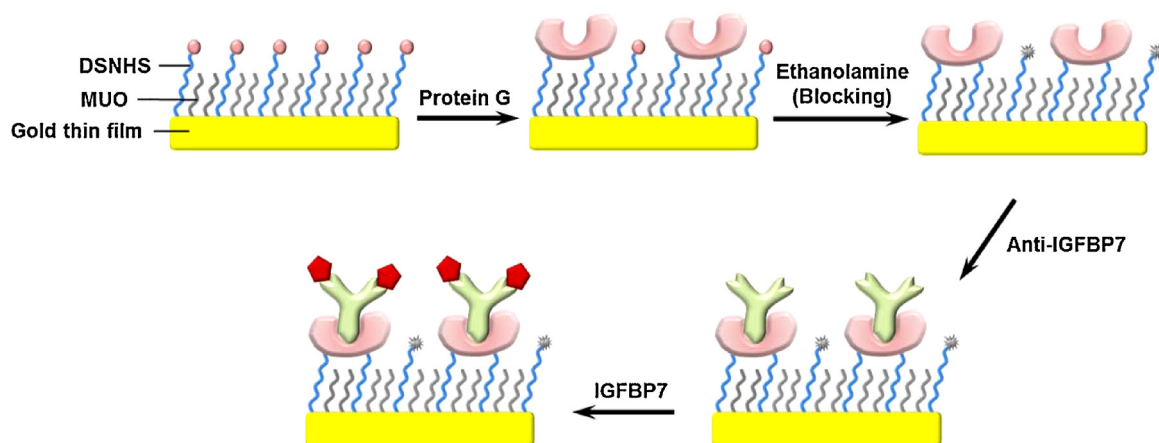


Fig. 1. Schematic diagram for the detection of IGFBP7 using a surface plasmon resonance biosensing technique. The scheme shows the use of a mixed SAM and protein G modified gold thin film substrate to immobilize anti-IGFBP7 proteins for the preparation of a sensing interface.

[34,35], which leads to the reduction of the non-specific binding and enhancement of sensitivity for IGFBP7 with a wide range of detection. The molar ratio between DSNHS and MUO was optimized to be 1:4 for the best coverage of anti-IGFBP7 (see Fig. S1 in Supporting Information).

The functionalized gold thin film with the mixed SAM was mounted on the SPR sensor and all the subsequent molecular interactions were monitored in real-time as shown in Fig. 2. After establishing a baseline in PBS, the diluted solution of protein G in PBS was pumped into the flow cell chamber to activate the sensor surface for immobilization. After injection for 20 min, the SPR signal was saturated, and the injection of protein G into the chamber was stopped. The non-covalently bound proteins were then removed by washing with a high ionic strength buffer. After a short injection of PBS, 1.0 M solution of ethanolamine hydrochloride was injected into the chamber for 5 min to deactivate the unreacted esters. A sudden increase and decrease in the signal at the beginning and end of the injection of ethanolamine hydrochloride was due to the drastic change in refractive index caused by replacing the ethanolamine solution with PBS. The immobilization of anti-IGFBP7 proteins to the protein G-coated surface was performed in the running buffer for 20 min. The injection time for the anti-IGFBP7 proteins was controlled by monitoring the signal, and the injection was stopped when the signal became saturated. This step was followed by washing the surface with PBS to remove any unbound

proteins. The results suggest that the immobilization of the anti-IGFBP7 on the SAM-modified gold thin film reached completion within 80 min due to its strong adsorption characteristics.

3.2. Detection of IGFBP7 standard protein by SPR biosensor

The standard IGFBP7 protein solution was diluted with PBS medium and the sensing performance of the SPR biosensor was investigated. To establish a calibration curve and explore the sensitivity of the SPR biosensor, changes in the SPR reflectance (ΔR) were determined after the exposure of IGFBP7 in the concentration range from 10 ng/ml to 1.0 μ g/ml. The detection of IGFBP7 was performed in PBS at a temperature of 25 °C and a flow rate of 50 μ l/min. A 10 min continuous injection of IGFBP7 with both reference and measuring channels was performed. After 10 min, the sample solution was replaced with running buffer and the dissociation phase was monitored. Fig. 3 shows the real-time changes in SPR signal as a function of concentration. When the sensor surface is exposed to the protein solutions, the SPR reflectance increased in typical runs, reaching equilibrium within approximately 10 min or less, after exposure to IGFBP7.

For the construction of a calibration curve, the sensor response was read after 20 min in buffer. An insufficient detection time results in a low coverage of IGFBP7 on the sensor surface and leads

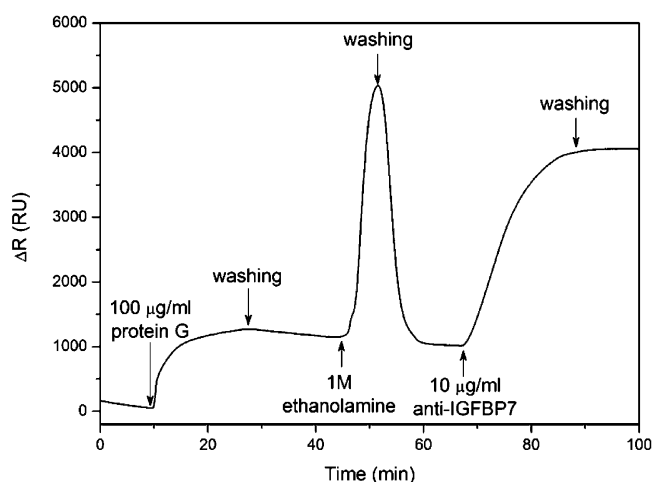


Fig. 2. Time-resolved SPR signal for the immobilization of anti-IGFBP7 proteins on the protein G-modified gold thin film.

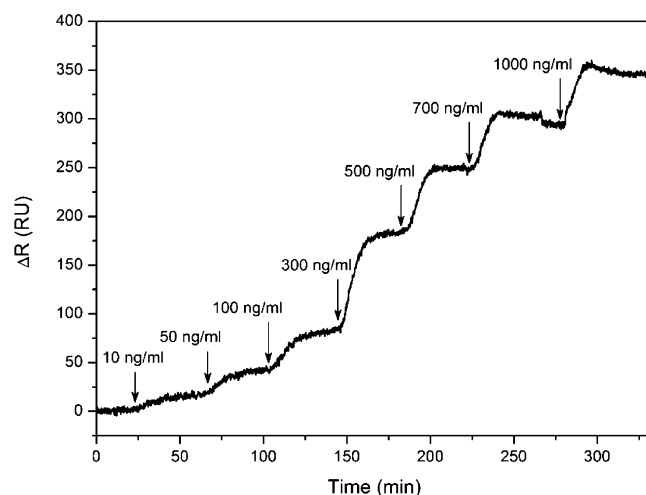


Fig. 3. Time-resolved change in reflectance (ΔR) near the SPR angle following the angle shifts measured for different concentrations of IGFBP7 in the range of 10 ng/ml to 1.0 μ g/ml.

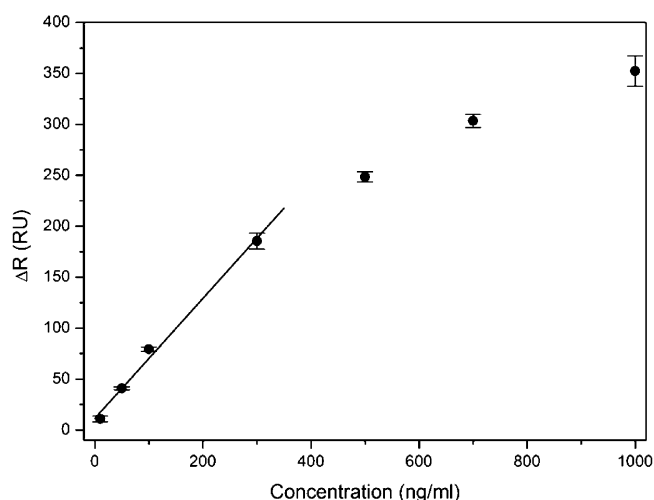


Fig. 4. The calibration curve for IGFBP7 as a function of concentration from 10 ng/ml to 1.0 μ g/ml. The error bar shown indicates the standard deviation from the mean ($n=3$).

to a lower sensitivity, whereas an over-long detection time may result in multiple layers of IGFBP7, leading to the blockage of binding sites and lower efficiency. Fig. 4 shows the calibration curves for the SPR biosensor utilizing anti-IGFBP7 proteins. The results were created using sensor responses from a minimum of three substrates with three replicas per substrate. Concerning the signal noise (the SPR reflectance of the thin film fluctuated within $\pm 0.2\%$ in aqueous media in the absence of IGFBP7), the limit of detection was determined to be 10 ng/ml, which is more sensitive than the biologically relevant range of concentrations. It can thus be concluded that the SPR biosensor for IGFBP7 would be applicable for use in biomedical diagnosis and monitoring.

It should be noted that the responses of the biosensor toward IGFBP7 are quite linear over the entire calibration range of 10 ng/ml to 300 ng/ml. At higher concentrations, the response of IGFBP7 to the sensor surface became saturated, leading to a nonlinear response and a plateau at concentrations above 1.0 μ g/ml. The reason for the non-linear response at higher IGFBP7 concentrations is that the receptor sites of the antibody were nearly saturated with IGFBP7 molecules and, as a result, only a slight change in reflectance occurred. That is, the operational amplitude (ΔR) initially increases in proportion to the concentration of IGFBP7, reaching a limiting value (≈ 350 RU).

3.3. Evaluation of specificity of SPR biosensor for IGFBP7

We also tested the specificity of the SPR sensor for IGFBP7 against in the presence of other biologically relevant molecules including interleukin derivatives including IL4, IL23, IL29, and IFG1, which are one of the predominant cytokines found in cell culture media, and which have a molecular weight similar to that of IGFBP7 [36–39]. Fig. 5 shows reflectance changes as a function of the concentration of each biomolecule. The reflectance change increases with increasing concentration of these molecules in the range of 10 ng/ml to 1.0 μ g/ml. Compared to the response for IGFBP7, the sensing performance to these molecules were similar to IGFBP7 at a low concentration range. At concentrations of 10 ng/ml and 50 ng/ml, the SPR signal of IGFBP7 is not significant against IL4, IL23 and IL29, and IFG1. In particular, both IL23 and IL29 cause a slightly greater change in SPR signal than that of IGFBP7. This suggests that at the low concentrations, these species can participate in competitive binding and interfere with the binding on the sensor surface with IGFBP7. Interestingly, for concentrations over

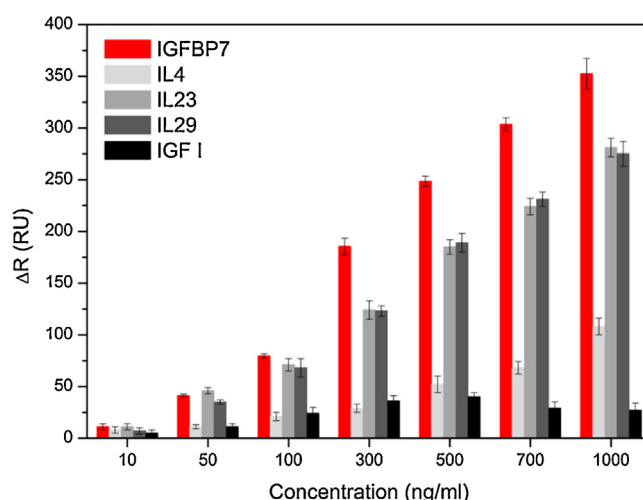


Fig. 5. SPR signal changes in ΔR of a sensor surface after exposure to concentrations ranging from 10 ng/ml to 1.0 μ g/ml of IGFBP7, IL4, IL23, IL29, and IFG1, respectively. The error bar shown is the standard deviation from the mean ($n=3$).

100 ng/ml, a higher signal change for IGFBP7 was found, over not only IL4 and IFG1 but also IL23 and IL29 species. In the case of IL4 and IFG1, small changes in SPR signal were observed at low and high concentrations as well.

It should be noted that in the overall graphical shape, the highest slope of linearity was observed in the calibration curve of IGFBP7 compared to that of other biomolecules (see Fig. S2 in Supporting Information). In other words, the slope for the SPR signal of IGFBP7 as a function of concentration is quite distinct with respect to IL23, IL29, IL4, and IFG1. Therefore, this SPR biosensor and the corresponding calibration curves show specificity for IGFBP7 over IL23, IL29, IL4, and IFG1.

3.4. Feasibility test of the SPR biosensor for IGFBP7 in cell culture media

In order to evaluate the analytical reliability and potential for applying the SPR biosensor, we further used the sensor surface to quantify IGFBP7 in cell culture media. The supernatant from human lung carcinoma A-549 cells was collected after cell culturing for 48 h

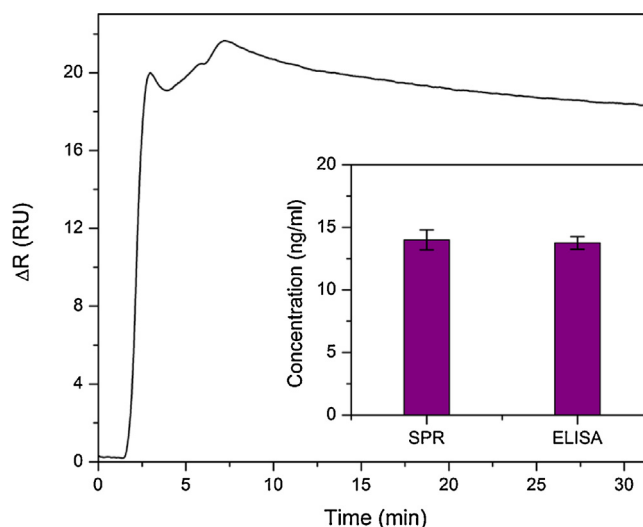


Fig. 6. Time-resolved change in ΔR measured for IGFBP7 in a supernatant obtained from human lung carcinoma A-549 cells. The inner graph is the quantification of IGFBP7 level by the SPR biosensor and by ELISA.

to investigate the feasibility of the technique for use in biomedical applications. The supernatant sample was directly analyzed by both ELISA and SPR. The real-time SPR signal for the media sample was obtained and the value was determined to be ca. 18 RU. Although the cell culture medium contained high concentrations of protein and glucose, no nonspecific interactions with the sensor surface were detected. In the linear range of the calibration curve in Fig. 4, the concentration of secreted IGFBP7 was estimated to be ca. 14 ng/ml, which was in good agreement with results obtained using ELISA (13.75 ± 0.5 ng/ml) within the error bar (Fig. 6). Overall, the proposed SPR biosensor is simple, convenient and rapid, with a wide detection range for IGFBP7, suggesting that it has considerable potential for use in the real-time monitoring of cellular secretions and other practical biomedical applications.

4. Conclusions

In conclusion, an SPR biosensor for the sensitive and selective detection and quantification of IGFBP7 using a mixed SAM in conjunction with a specific receptor on a gold thin film is described. The basis for the analytical method involves the specific and selective interaction of IGFBP7 with an anti-IGFBP7 protein by the mixed SAM and protein G-modified gold thin film, which results in changes in the SPR profile. The limit of detection was determined to be 10 ng/ml and a wide linear response was observed for IGFBP7 concentrations in the range of 10–300 ng/ml. In terms of specificity, the slope of the linearity in the sensing performance for IGFBP7 is definitely distinguishable from other relevant biomolecular species including IL4, IL23, IL29, and IFG1. Furthermore, the SPR biosensor was also feasible for use in the analysis of IGFBP7 in cell culture media without interference by other proteins, thus making it a viable alternative method to the currently used ELISA. Based on the results of a wide range of sensitivity, specificity toward biologically relevant molecules, and feasibility of use in the presence of other molecules that are secreted by cells secretion, we conclude that the proposed SPR biosensor for IGFBP7 can be applied to biomedical fields such as diagnostic monitoring.

Competing interest

The authors declare that they have no competing interests.

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Appendix A. Supplementary data

Supplementary data associated with this article can be found, in the online version, at <http://dx.doi.org/10.1016/j.colsurfb.2014.10.037>.

References

- [1] J. Nussbeck, A. Ishida-Yamamoto, M. Bidder, D. Fuchs, K. Eckl, H.C. Hennies, N. Sagiv, A. Gat, M. Gini, I. Filip, H. Matz, I. Goldberg, B. Meilik, D. Aberdam, A. Gilhar, E. Sprecher, *J. Invest. Dermatol.* 131 (2011) 1767.
- [2] M. Kutsukake, R. Ishihara, M. Yoshie, H. Kogo, K. Tamura, *Mol. Hum. Reprod.* 13 (2007) 737.
- [3] K. Tamura, K. Hashimoto, K. Suzuki, M. Yoshie, M. Kutsukake, T. Sakurai, *Eur. J. Pharmacol.* 610 (2009) 61.
- [4] M.G. Vizioli, M. Sensi, C. Miranda, L. Cleris, F. Formelli, M.C. Anania, M.A. Pierotti, A. Greco, *Oncogene* 29 (2009) 3835.
- [5] Z.-K. Liu, R.-C. Wang, B.-C. Han, Y. Yang, J.-P. Peng, *PLoS ONE* 7 (2012) e45224.
- [6] E. Smith, A.R. Ruszkiewicz, G.G. Jamieson, P.A. Drew, *Br. J. Cancer* 110 (2014) 775.
- [7] D.D. Shersher, M.S. Vercillo, C. Fhied, S. Basu, O. Rouhi, B. Mahon, J.S. Coon, W.H. Warren, L.P. Faber, E. Hong, P. Bonomi, M.J. Liptay, J.A. Borgia, *Ann. Thorac. Surg.* 92 (2011) 1805.
- [8] Z. Wang, Z. Wang, Z. Liang, J. Liu, W. Shi, P. Bai, X. Lin, R. Magaye, J. Zhao, *J. OncoTargets Ther.* 6 (2013) 1437.
- [9] W.F.M.A. Kotb, I. Petersen, *Pathol. Res. Pract.* 208 (2012) 1.
- [10] F. Aregger, D.E. Uehlinger, J. Witowski, R.A. Brunisholz, P. Hunziker, F.J. Frey, A. Jörres, *Kidney Int.* 85 (2014) 909.
- [11] K. Kashani, A. Al-Khafaji, T. Ardiles, A. Artigas, S.M. Bagshaw, M. Bell, A. Bino-rac, R. Birkhahn, C.M. Cely, L.S. Chawla, D.L. Davison, T. Feldkamp, L.G. Forni, M.N. Gong, K.J. Gunnerson, M. Haase, J. Hackett, P.M. Honore, E.A.J. Hoste, O. Joannes-Boyau, M. Joannidis, P. Kim, J.L. Koyner, D.T. Laskowitz, M.E. Lissauer, G. Marx, P.A. McCullough, S. Mullaney, M. Ostermann, T. Rimmelé, N.J. Shapiro, A.D. Shaw, J. Shi, A.M. Sprague, J.-L. Vincent, C. Vinsonneau, L. Wagner, M.G. Walker, R.G. Wilkerson, K. Zacharowski, J.A. Kellum, *Crit. Care* 17 (2013) R25.
- [12] M. Meersch, C. Schmidt, H.V. Aken, S. Martens, J. Rossaint, K. Singbartl, D. Görl-lich, J.A. Kellum, A. Zarbock, *PLoS ONE* 9 (2014) e93460.
- [13] D. Chen, B.K. Yoo, P.K. Santhekadur, *Clin. Cancer Res.* 17 (2011) 6693.
- [14] D. Chen, A. Siddiq, L. Emdad, D. Rajasekaran, R. Gredler, X.-N. Shen, P.K. San-thekadur, J. Srivastava, C.L. Robertson, I. Dmitriev, E.A. Kashentseva, D.T. Curiel, P.B. Fisher, D. Sarkar, *Mol. Ther.* 21 (2013) 758.
- [15] E.S. Schemhammer, J.M. Holly, D.J. Hunter, M.N. Pollak, S.E. Hankinson, *Endocr. Relat. Cancer* 13 (2006) 583.
- [16] A. Diamandi, J. Mistry, R.G. Krishna, J. Khosravi, *J. Clin. Endocrinol. Metab.* 85 (2000) 2327.
- [17] G. Parikh, M. Varadinova, P. Suwandhi, T. Araki, Z. Rosenwaks, L. Poretsky, D. Seto-Young, *Horm. Metab. Res.* 42 (2010) 754.
- [18] H. Shahjee, N. Bhattacharyya, G. Zappala, M. Wiench, S. Prakash, M.M. Rechler, *Growth Horm. IGF Res.* 18 (2008) 188.
- [19] M. Swain, R. Thiupathi, B. Krishnarajuna, E.M. Eaton, M.M. Kibbey, S.A. Rosen-zweig, H.S. Atreya, *Chem. Commun.* 46 (2010) 216.
- [20] E.B. Hunziker, E. Kapfinger, J. Martin, J. Buckwalter, T.I. Morales, *Osteoarthritis Cartilage* 16 (2008) 185.
- [21] D.S. Daly, A.M. White, S.M. Varnum, K.K. Anderson, R.C. Zangar, *BMC Bioinform.* 6 (2005) 1.
- [22] K.M. Higgins, M. Davidian, G. Chew, H. Burge, *Biometrics* 54 (1998) 19.
- [23] T.-H. Chou, C.-Y. Chuang, C.-M. Wu, *Cytokine* 51 (2010) 107.
- [24] G. Stybayeva, M. Kairova, E. Ramanculov, A.L. Simonian, A. Revzin, *Colloids Surf. B* 80 (2010) 251.
- [25] H. Šipová, V. Ševců, M. Kuchař, J.N. Ahmad, P. Milulecky, R. Osička, P. Malý, J. Homola, *Sens. Actuators B* 174 (2012) 306.
- [26] N. Tuleuova, C.N. Jones, J. Yan, E. Ramanculov, Y. Yokobayashi, A. Revzin, *Anal. Chem.* 82 (2010) 1851.
- [27] S. Hong, S. Lee, I. Choi, Y.I. Yang, T. Kang, J. Yi, *Colloids Surf. B* 101 (2013) 266.
- [28] S. Hong, S. Park, J. Park, J. Yi, *Colloids Surf. B* 112 (2013) 415.
- [29] B. Sepúlveda, A. Calle, L.M. Lechuga, G. Armelles, *Opt. Lett.* 31 (2006) 1085.
- [30] M. Iga, A. Seki, K. Watanabe, *Sens. Actuators B* 101 (2004) 368.
- [31] R.G. Chapman, E. Ostuni, L. Yan, G.M. Whitesides, *Langmuir* 16 (2000) 6927.
- [32] J. Lahiri, L. Isaacs, J. Tien, G.M. Whitesides, *Anal. Chem.* 71 (1999) 777.
- [33] R.E. Holmlin, X. Chen, R.G. Chapman, S. Takayama, G.M. Whitesides, *Langmuir* 17 (2001) 2841.
- [34] J.C. Love, L.A. Estroff, J.K. Kriebel, R.G. Nuzzo, G.M. Whitesides, *Chem. Rev.* 105 (2005) 1103.
- [35] C. Cao, J.P. Kim, B.W. Kim, H. Chae, H.C. Yoon, S.S. Yang, S.J. Sim, *Biosens. Bioelectron.* 21 (2006) 2106.
- [36] S.-M. Kim, S.-H. Moon, Y. Lee, G.J. Kim, H.-M. Chung, Y.-S. Choi, *Stem Cells Dev.* 22 (2013) 3025.
- [37] H.E. Gruber, G.L. Hoelscher, J.A. Ingram, D.S. Morton, E.N. Hanley Jr., *Connect. Tissue Res.* 54 (2013) 432.
- [38] M.F. Gude, J. Frystyk, A. Flyvbjerg, J.M. Bruun, *Growth Horm. IGF Res.* 22 (2012) 200.
- [39] Y.H. Noh, Y.S. Yim, D.H. Kim, M.W. Lee, D.S. Kim, H.R. Kim, S.H. Lee, H.W. Chueh, S.J. Choi, W.I. Oh, Y.-S. Yang, H.L. Jung, K.H. Yoo, K.W. Sung, H.H. Koo, *Pediatr. Hematol. Oncol.* 28 (2011) 682.