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SPR imaging biosensor for the quantitation of fibronectin concentration in blood samples

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ABSTRACT

The purpose of this study was presentation of a new biosensor capable of determination of fibronectin. This biosensor was based on the specific interaction of anti-fibronectin antibody produced in rabbit with fibronectin. The surface plasmon resonance imaging (SPRI) technique was used as a detecting method. Optimization and characterization properties of the biosensor were studied. The determination of fibronectin concentration in natural samples was done. The results were compared with a reference method (Enzyme-Linked Immunosorbent Assay-ELISA). The analytically useful dynamic response range of biosensor is between 5 and 400 ng mL^{-1}. The detection limit is 1.5 ng mL^{-1} and limit quantification is 5 ng mL^{-1}. The proposed SPRI biosensor showed good selectivity for potential interferences. It was applied to determine fibronectin concentrations in plasma of healthy donors and of patients after thermal injury. Good correlations between results obtained using the SPRI biosensor and ELISA test (correlation coefficients for healthy donors 0.996, for patients 0.984) were obtained. The average fibronectin concentration of healthy donors was 140.5 ± 24.6 μg mL^{-1} and the average fibronectin concentration of patients was 601.5 ± 72.1 μg mL^{-1}, which was in agreement with results obtained by other investigators. The obtained results indicate that the developed biosensor may be a candidate for monitoring fibronectin concentration in blood samples.

1. Introduction

Surface plasmon resonance imaging (SPRI) was first demonstrated in the late 1980s [1,2]. Since then, SPRI has found rapid increasing research interests. Extensive research work on SPR imaging has been conducted by Corn et al. [3]. In the literature we can find various SPRI systems, for example: Multi-parametric Surface Plasmon Resonance (MP-SPR) [4], Localized Surface Plasmon Resonance (LSPR) [5] and spectral surface plasmon resonance (λSPR) [6].

This technique measures the refractive index changes in the vicinity of thin metal layers in response to biomolecular interactions. The refractive index is proportional to the amount of immobilized molecules on the metal surface. The label-free nature of SPRI makes it an attractive alternative technique to others, in which labels have to be used. The labeling process can cause the loss of functional properties of biomolecules (functional proteomics).

SPRI biosensor allows the real-time biomolecules detection. SPRI experiment may be exploited to monitor interaction between molecules and determining the kinetics of interaction [7–9].

The combination of SPRI technique with the biosensor is a sensitive, quantitative and rapid measurement method with abundant applications in the bioanalytical field [10–15].

The analytically active layer (receptor) of biosensor is formed with biological material being immobilized on a suitable support. Receptor type affects the sensitivity and selectivity with respect to the test substance. The antibody or inhibitor may be used as recognition elements in SPRI biosensor. The numerous examples of successful use of SPRI biosensors for the determination of diagnostically significant biomolecules have been reported [6–8]. The SPRI biosensor may be one of the competitive techniques in

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comparison to the ELISA assay in the determination of diagnostically significant biologically active species [16,17].

Fibronectin (FN) is one of the biologically active species which may be important as a potential biomarker. It is a glycoprotein that is an essential component of the extracellular matrix (ECM). FN is present in plasma and in various types of tissue and plays important role in cell adhesion, growth, migration, differentiation, wound healing and embryonic development [18,19].

The basic structural unit of FN is a dimer composed of two similar but not always identical polypeptide chains of similar molecular weight of about 250 kDa. Each chain of fibronectin is composed with repeated motifs of the amino acid sequence designated as type I, II and III. There are 12 modules of the type I, two of type II and 15–17 of type III in one subunit. The modules are distributed irregularly and form a mosaic structure of the protein [18,19].

Fibronectin in mammals coexists in the multiple molecular forms. It exists in a soluble form – plasma (PFN), and two insoluble forms: bound to the cell membrane and present in the extracellular matrix as cross-linker. According to Hynes [20], PFN is a globular protein consisting of independently folded globular domains and with a relatively compact conformation. Insoluble FN is in extended conformation and may be assembled into fibronectin fibrils [20].

FN soluble form occurs not only in plasma, but in other biological fluids: amniotic fluid [21], cerebrospinal fluid [22], saliva [23,24]. The soluble fibronectins are synthesized by hepatocytes while the insoluble forms are produced by fibroblasts, macrophages, endothelial cells and epithelial cells [18].

Numerous studies have shown that the fibronectin deposition into the extracellular matrix and the turnover of FN is a dynamic process. The steady state exists between assembly of ECM, its remodeling and degradation. FN fibrils provides the matrix with stability and rigidity [19].

The change in expression of fibronectin, organization, degradation and its concentration in body fluids has been reported in various disease conditions. The levels of fibronectin increase in plasma of patients with gastric cancer [25,26], colorectal cancer [27], breast cancer [28], bladder cancer [29], rheumatic disease [30], ischemic heart disease [31], collagen vascular diseases, diabetes mellitus [32]. Fibronectin detected in cerebrospinal fluid and serum may be a potential epilepsy biomarker [22].

FN concentration in plasma has low values in septic patients compared to healthy subjects and patients with different non-infectious pathologies and FN appears to act as a marker of sepsis [33].

The most common methods used for determination of fibronectin and its degradation products are immunohistochemistry [34,35] and enzyme-linked immunosorbent assays (ELISA) [22,25,29,31,32] respectively.

The fibronectin concentration in plasma was also determined by laser nephelometry [33,36] or immunoturbimetric method [30,37]. A competitive radioimmunoassay (CRIA) for quantitating determination of human plasma fibronectin levels has been described [38]. The presence of cellular FN was determined in the plasma using a time-resolved fluorescence immunoassay (TRFIA) [39,40].

The classic version of SPR technique was used for determination of fetal fibronectin [41]. The interaction of fibronectin and the cells was studied using SPRI technique. This technique makes it possible to monitor cellular modifications of FN in relation to the extracellular matrix forms in real-time [42].

The aim of this work was to develop SPRI method for the determination of fibronectin concentration in biological fluids. The biosensor on the basis of fibronectin interaction with specific antibody was constructed. Next, the analytical parameters of SPRI biosensor were optimized and the biosensor's ability to determine fibronectin in biological samples was tested.

2. Experimental materials and methods

2.1. Reagents

Fibronectin from human plasma (lyophilized powder) as standard, anti-fibronectin antibody produced in rabbit, collagen type IV, glycoprotein GPIIb/IIIa, human albumin, bovine serum albumin (BSA), cysteamine hydrochloride, *N*-ethyl-*N*′-(3-dimethylaminopropyl) carbodimide (EDC) (Sigma, Steinheim, Germany, http://www.sigmaaldrich.com/safc/facilities/steinheim-germany.html), N-hydroxysuccimide (NHS), (Aldrich, Munich,Germany,http://www.sigmaaldrich.com/germany.html), photopolimer ELPEMER SD 2054 and hydrophobic protective paint SD 2368UV SG-DG (Peters, Kempen, Germany, http://www.peters.de) were used, as well as absolute ethanol, acetic acid, hydrochloric acid, sodium hydroxide, sodium chloride, sodium carbonate, sodium acetate, (POCh, Gliwice, Poland, http://www.poch.com.pl/). HBS-ES buffer pH = 7.4 (0.01 M HEPES, 0.15 M sodium chloride, 0.005% Tween 20, 3 mM EDTA), Phosphate Buffered Saline (PBS) pH = 7.4, carbonate buffer pH = 8.5 (BIOMED, Lublin, Poland, http://www.biomed.lublin.pl) were used as received. Aqueous solutions were prepared with milliQ water (Simplicity® Millipore). Argon N 5.0 with a content Ar ≥ 99,999% was used (AIR LIQUIDE Polska Sp.z o.o., Poland). ELISA kit for fibronectin determination (Abcam, Cambridge, United Kingdom, http://www.abcam.com) was used.

2.2. Biological samples

All blood samples were taken from children with thermal injuries during the hospital admission to the L. Zamenhof Children's Clinical Hospital of the Medical University of Bialystok, Pediatric Surgery Department (Bialystok, Poland). Patients were aged 9 months to 14 years old. The blood samples were collected from the median cubital vein using EDTA as an anticoagulant. Also, the blood samples of the control group were taken from healthy people (age 18–35 years old), who are Honorary Blood Donors of the Regional Blood Donor Centre of Bialystok, Poland.

Two milliliters of blood was centrifuged ($1000 \times g$) for over 15 min and filtered 3-times for the separation of blood plasma from the cells. The blood plasma samples were frozen and stored at −70 °C until their further use. The samples were diluted 1000–5000 times with PBS buffer directly prior to measurement.

The study had the approval of the Ethics Committee of the Medical University in Bialystok and all patients gave informed consent.

2.3. Procedures

2.3.1. Chip preparation

Basis of the biosensor for fibronectin determination was prepared as described in the previous paper [37]. The glass chips were covered with gold (50 nm on a 1 nm thick chromium layer). Next, gold surface was covered with photopolymer and hydrophobic paint. An array of 9 × 12 free gold surfaces was made – Fig. 3. The nine different solutions can be simultaneously applied on chip. Twelve independent measurements can be made for each solution, so twelve individual SPRI signals for each sample can be obtained.

2.3.2. Antibody immobilization

The chip surface was rinsed with absolute ethanol and water, dried in a stream of argon and was immersed in 20 mM cysteamine ethanolic solutions for a minimum of 18 h. The chips with immobilized linker were rinsed and dried as described above. In order to immobilize antibody,50 μL of antibody solution (4 μg mL^{-1}, designated from the experiments for optimization of antibody concentration) was mixed with 250 μL of

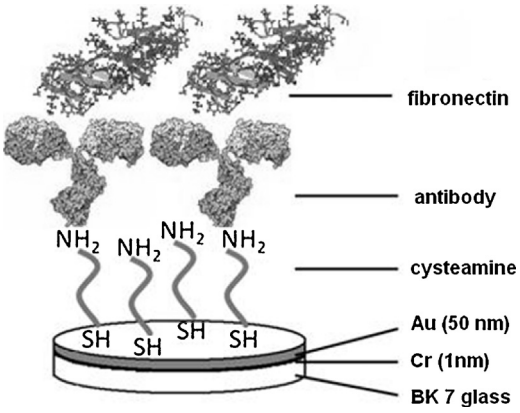

Fig. 1. The schematic illustration of the sensor active part.

50 mM N-hydroxysuccinimide (NHS), 250 μL of 200 mM *N*-ethyl-*N*′-(3-dimethylaminopropyl) carbodiimide (EDC) and 100 μL of carbonate buffer (pH = 8.5) and placed on the amine-modified surface. The chip prepared in this manner was incubated at 37 °C for 1 h. After this time, the surface of the prepared biosensor was rinsed five times with PBS buffer. Then, 1 mg/mL BSA in PBS was placed on the chip for 10 min to minimize nonspecific adsorption on the surface and it was rinsed with PBS buffer. The mixture of sodium acetate (50 μL, 5 mM), NHS (250 μL of 50 mM), EDC (250 μL of 200 mM) and carbonate buffer (100 μL, pH = 8.5) was used to deactivate any remaining amine groups. Finally, the chip was rinsed with PBS buffer and water. The schematic illustration of the sensor active part is presented on Fig. 1.

SEM pictures were used to confirm the creation of subsequent layers: gold, thiol (cysteamine), antibody and immobilized fibronectin. Fig. 2 shows a representative picture of the SEM measurements. Pictures were taken of bare gold (Fig. 2a), cysteamine (Fig. 2b), antibody (Fig. 2c) and immobilized fibronectin (Fig. 2d). The presented SEM pictures confirm that the described stages of the creation of each layer on the biosensor surface have really taken place. This may be concluded on the basis of the creation of different structures after each stage.

2.3.3. SEM measurements

The Scanning Electron Microscopy (SEM) measurements were performed with a commercial SEM Phenom ProX equipped with a CeB_6 electron source.

Microscopic examination was performed with using the accelerating voltage of 10 kV at magnifications of 5000× and using the backscattered electron detector (BSE). Fig. 2 shows representative SEM images of the subsequent layers on the chip surface.

2.3.4. SPRI measurement

SPRI measurements were performed on a home-made apparatus which had been successfully used and described previously [16,17,43]. The apparatus (Fig. 3) consists of a HeNe laser (JDS Uniphase, Edmund Industrial Optics, USA), two glass lenses L1 (f = 3 mm) and L2 (f = 300 mm), two polarizers (P1 and P2), a mirror, a prism and a CCD camera (QICam, QImaging, Canada). NIH Image J version 1.42 software was used for evaluation of the SPRI images in 2D form. The biosensor preparation was described in the preceding paragraphs.

The first step was optimization of SPR angle. The angle at which there was the best contrast between active site and background was selected. The measurements were performed at a fixed angle of incident light and the reflectivity was simultaneously measured across an entire chip surface. The signal was measured on the basis of images registered by CCD camera. The images were taken after antibody immobilization and after interaction between antibody and fibronectin. All measurements are performed under stationary conditions. The biosensor consists of 12 spots for 9 different samples. The contrast values obtained for all pixels across a particular sample single spot were integrated. A background correction was applied i.e. some of the places on the biosensor covered with PBS buffer were used as a control. Non-specific binding was monitored by measuring the SPRI signal in place on the chip without the receptor (ligand). Minimization of the non-specific binding was obtained by preparing samples in PBS buffer and by placing BSA in PBS buffer on the chip. The SPRI signal, which was proportional to the mass of entrapped fibronectin, was obtained as the difference between the signals before and after interaction with the analyzed sample, for each spot separately.

The biosensors used can be regenerated [44]. The regeneration of biosensors is not used during the measurements as a part of the procedure, because that stationary approach was used.

2.4. Statistical analysis

All results were given as mean ± standard deviation. Student's *t*-test was used for statistical analysis. The data obtained by SPRI biosensor and ELISA for natural samples was analyzed using Pearson's correlation. A value of $p < 0.05$ was considered significant.

3. Results and discussion

3.1. Influence of antibody concentration on the analytical signal of fibronectin

The usefulness of biosensor was determined by the receptor concentration and ought to be optimized. The rabbit anti-fibronectin antibody was used as a receptor. The purpose of this investigation was to find the optimal antibody concentration. Experiments were performed at pH = 7.4 and at a constant fibronectin concentration 500 ng mL^{-1} was used. Antibody concentration varied between 0.5 and 7.0 μg mL^{-1}. The antibody solutions were transferred on to the chip, which had previously been modified with cysteamine thiol. The chip was then treated with the fibronectin solution by 10 min in the room temperature. After the interaction, the chip was rinsed with HBS-ES buffer and water (at least 10 times) and dried, and the SPRI measurement was then performed. The results are shown in Fig. 4. The obtained curve was of the Langmuir isotherm type. Gradual formation of a plateau was the evidence of saturation of the biosensor surface with antibody. Further increase in antibody concentration did not cause the increase in surface antibody concentration. Correct SPRI measurement should be performed with the receptor concentration corresponding to this plateau. On the basis of curve in Fig. 4, a concentration 4 μg mL^{-1} was selected as the optimal antibody concentration for further investigations.

3.2. Analytical response of the sensor to fibronectin concentration. Calibration curve

The calibration curve for fibronectin determination with a biosensor was performed in optimized conditions. The antibody concentration was 4 μg mL^{-1} and pH = 7.4.

The response of the analytical SPRI signal for fibronectin was measured within a range of its concentration between 5 and 1000 ng mL^{-1}. The results are shown in Fig. 5. The curve was of typical of Langmuirian type. The plateau of the curve corresponded to saturation of active sites of the biosensor. The linear section of the curve (Fig. 5) was in range 5–400 ng mL^{-1} ($R^2 = 0.9938$) and was useful for analytical purposes.

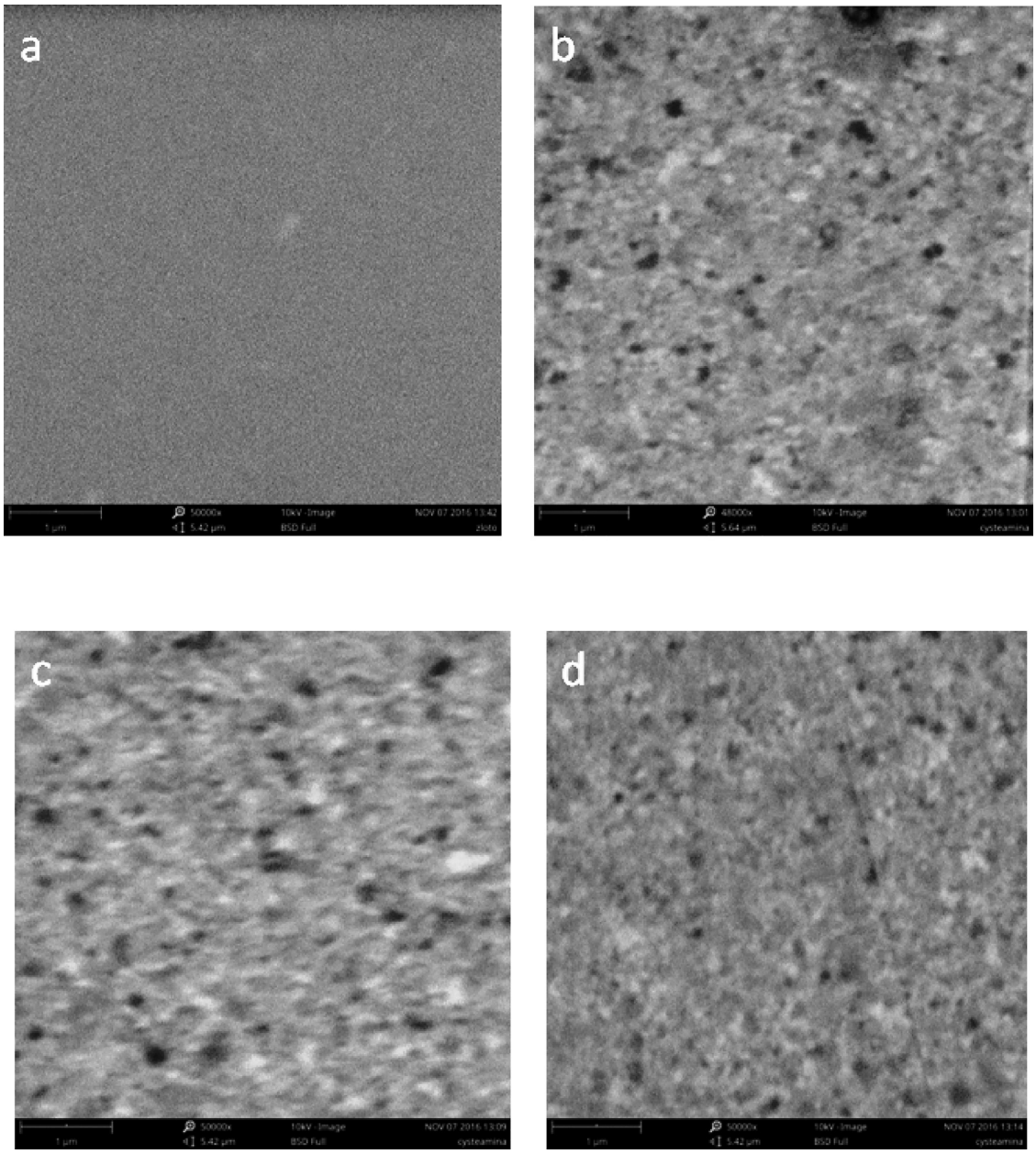

Fig. 2. SEM images of the subsequent layers on the gold chip surface, a) the pure gold; b) the cysteamine layer; c) the antibody layer; and d) the fibronectin bound to the chip.

3.3. Recovery and precision of the developed method

Four series of measurements were performed for the determination of recovery, precision, and limits of detection (LOD) and quantification (LOQ). The first series was 'blank', i.e. performed without a fibronectin spike, other three series were performed with fibronectin spikes of 5.0, 200.0 and 400.0 ng mL^{-1} respectively. The results are shown in Table 1.

Both precision and recovery were typical for this kind of determination, and they were satisfactory. The recoveries of fibronectin spikes appeared in range 101–104%. The LOD calculated on the 3SD of the blank (PBS buffer) and was equal to 1.5 ng mL^{-1}. The LOQ calculated on the 10 SD of the blank and it was equal to 5.0 ng mL^{-1}.

Recovery of the developed method was also checked by the addition of standard solution of fibronectin (100 ng mL^{-1}) into nine different samples of human plasma 1000 fold diluted (final con-

Table 1
Recovery and precision of the developed method.

Series	Number of measurements	Spiked [ng mL^{-1}]	Found [ng mL^{-1}]	SD [ng mL^{-1}]	CV [%]	Recovery [%]
1	24	0	0	0.5[a]		–
2	24	5.0	5.2	0.3	6.0	104
3	24	200.0	203.7	3.2	1.6	102
4	24	400.0	405.3	4.8	1.2	101

[a] Standard deviation of the blank (buffer).

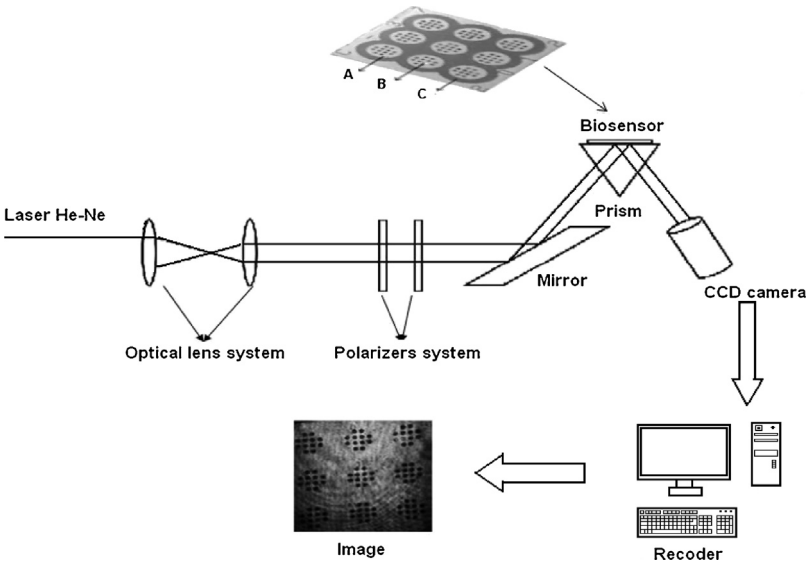

Fig. 3. The schematic diagram of the apparatus and the illustration of used biosensor (A-photopolymer, B-free gold surface, C-hydrophobic paint).

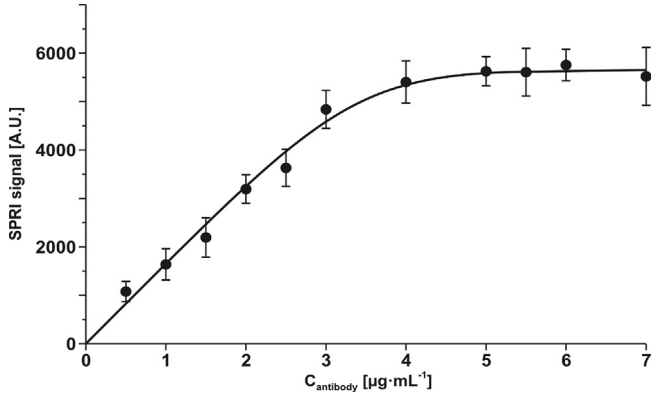

Fig. 4. Dependence of SPRI signal (Arbitrary Units) on rabbit anti-human fibronectin antibody concentration. Fibronectin concentration: 500 ng mL^{-1}. pH = 7.4. Error bars were calculated for 12 independent measurements for each concentration at a 95% confidence level.

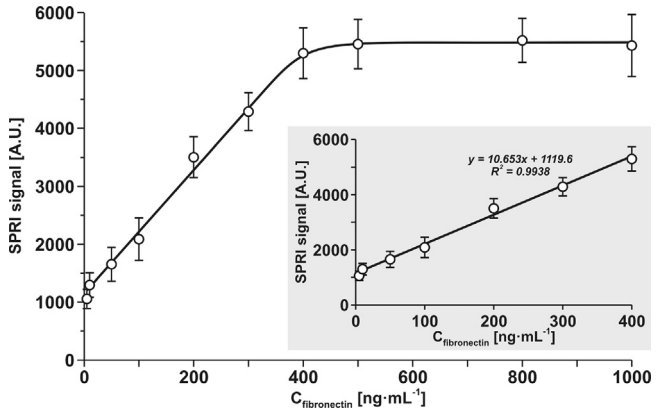

Fig. 5. Dependence of the SPRI signal (Arbitrary Units) on fibronectin concentration. Rabbit anti-human fibronectin antibody concentration: 4 µg mL^{-1}. pH = 7.4. Error bars were calculated for 12 independent measurements for each concentration at a 95% confidence level.

centration was about 100 ng mL^{-1}). The samples were mixed in equal volumes with twice higher concentrations. The results are presented in Table 2.

Table 2
Recoveries of fibronectin in plasma.

No of samples	Fibronectin concentration without spike [ng mL^{-1}]	Added standard concentration [ng mL^{-1}]	Determined [ng mL^{-1}]	Recovery [%]
1	125.3 ± 1.5	100	229.1 ± 2.1	102
2	145.1 ± 2.1	100	253.2 ± 2.6	103
3	157.1 ± 1.7	100	269.3 ± 2.5	105
4	168.6 ± 2.0	100	266.9 ± 1.9	99
5	118.9 ± 2.4	100	220.9 ± 2.4	101
6	103.7 ± 1.9	100	207.0 ± 2.4	101
7	101.4 ± 2.5	100	200.7 ± 1.9	100
8	145.7 ± 2.8	100	244.2 ± 1.5	99
9	146.6 ± 2.2	100	251.8 ± 1.7	102

The differences of determined concentrations were not statistically significant $p < 0.05$. Recoveries were within the range of 99–105% and showed no significant matrix effect.

3.4. Selectivity of the developed method

The selectivity of the developed method is a crucial factor for its potential application. In order to check if the interaction between antibody and fibronectin was selective, the chip with immobilized antibody was treated with mixtures of fibronectin with potential interferents. The albumin, collagen type IV, laminin, heparin and glycoprotein GPIIB/IIIA were investigated as potential interferents.

Fibronectin concentration was constant and selected as 100 ng mL^{-1}, i.e. the value of the dynamic response range of the method. The excess of interferents varied from 1:1 to 1:100. The results are shown in Table 3.

No significant influence of these substances on fibronectin determination was observed at 100-fold excess. Thus, the selectivity of the developed biosensor was confirmed. Applied antibody as a natural receptor has excellent molecular recognition capabilities and high affinity to fibronectin and antibody- fibronectin complex has good stability in measuring conditions.

3.5. Determination of fibronectin in biological samples

The amount of fibronectin in biological samples was determined using developed SPRI biosensor. The tests were performed in nine samples of plasma from healthy volunteers and from patients after

Table 3
Selectivity of fibronectin determination with the developed method in the presence of potential interferents: albumin, collagen IV, laminin-5, heparin, glycoprotein GPIIB/IIIA (n = 12).

Potential Interferent	Fibronectin concentration [ng mL^{-1}]	Excess over fibronectin	Measured fibronectin concentration [ng mL^{-1}]	SD [ng mL^{-1}]	Recovery [%]
–	100	–	100.9	1.5	101
		1:1	104.2	2.8	104
Albumin	100	1:10	97.2	3.1	97
		1:100	90.9	5.6	91
		1:1	107.6	1.9	108
Collagen IV	100	1:10	91.2	3.5	92
		1:100	94.1	2.9	94
		1:1	103.6	3.1	104
Laminin-5	100	1:10	102.1	2.4	102
		1:100	100.5	4.1	101
		1:1	108.4	4.0	108
Heparin	100	1:10	107.3	2.1	107
		1:100	103.9	2.8	104
		1:1	105.6	2.4	106
Glycoprotein GPIIB/IIIA	100	1:10	98.4	3.2	98
		1:100	99.6	4.1	100

Table 4
Concentration of fibronectin in human blood plasma of patients after thermal injury and healthy volunteers measured by the developed method.

	Fibronectin concentration [μg mL^{-1}]	
	Healthy donors	Patients after thermal injury
1	180.4 ± 1.9	473.0 ± 2.7
2	101.4 ± 2.5	865.5 ± 1.4
3	168.6 ± 1.5	595.9 ± 3.1
4	108.1 ± 2.4	563.0 ± 2.6
5	208.0 ± 1.5	591.4 ± 2.4
6	107.8 ± 2.1	624.3 ± 2.6
7	125.3 ± 1.7	563.8 ± 1.9
8	118,9 ± 2.4	621.1 ± 1.9
9	145.7 ± 2.8	515.6 ± 2.5
Mean value ± confidence limit	140.5 ± 24.6	601.5 ± 72.1
Range	101.4–208.0	473.0–865.5
Literature mean value	88.6 ± 22.2 [27]	
	94.0 ± 90.9 [45]	
	181.4 ± 72.8 [22]	
	221.0 ± 32.5 [46]	

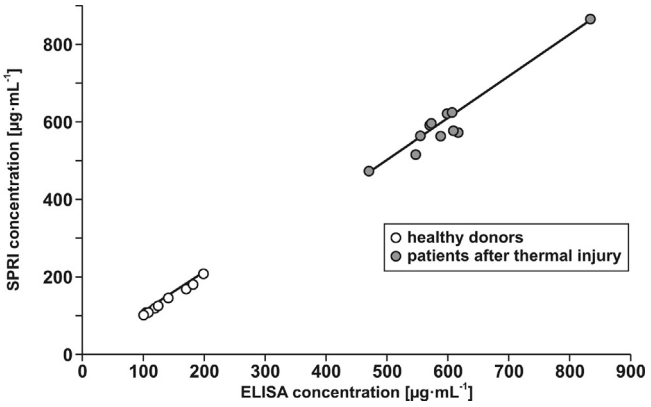

Fig. 6. The linear correlation between the results of fibronectin concentration in the plasma of (○) healthy donors, (●) patients after thermal injury, obtained by the SPRI methods and ELISA.

thermal injury. The aim of this experiment was to verify the suitability of the developed method for the analysis of typical real samples. The determination was performed as described in previous sections. The fibronectin concentration was evaluated on the basis of calibration curve (see Fig. 5). The results are shown in Table 4.

For healthy volunteers the mean value of fibronectin concentration was as 140.5 ± 24.6 μg mL^{-1} and for patients after thermal injury it was 601.5 ± 72.1 μg mL^{-1}. The various mean values of fibronectin concentration were reported in the literature (Table 3) and the results obtained by SPRI methods for healthy volunteers were located in this range. The fibronectin concentration of patients was higher than of healthy volunteers. However, in order to determine the role of fibronectin in processes occurring in body after thermal injury the studies should be extended. Further studies on larger group of patients should be performed and the patients should be classified by depth, mechanism of injury, extent, and associated injuries.

In order to validate the developed method, comparative determination of fibronectin concentration in the same samples by the commercial ELISA kit was performed. The results are shown in Fig. 6. The mean value of fibronectin concentration obtained by ELISA test was 138.8 ± 23.8 μg mL^{-1} (range: 100.5–198.9 μg mL^{-1}) of healthy volunteers and 593.7 ± 64.4 μg mL^{-1} (range: 470.5–833.9 μg mL^{-1}) of patients after thermal injury. Comparison between measurements of fibronectin concentration by SPRI biosensor and ELISA test showed a good agreement. The significant Pearson correlation coefficient value of 0.996 ($p < 0.001$) for healthy volunteers and 0.984 ($p < 0.001$) for patient confirmed to be a very strong positive correlation between the results obtained by two methods.

ELISA has been the preferred method for the determination of fibronectin concentration. Although it is a reliable technique with good sensitivity and selectivity, it is relatively time- and labor-intensive. SPRI may be an alternative technique to traditional immunobased assay like ELISA. Both methods have advantages but also disadvantages. For several analytical aspects namely sensitivity and specificity SPR has been found to be quite comparable to ELISA. In SPR, the fibronectin concentration can be detected in real time and using only one antibody. In comparison to ELISA, this technique does not require labels or the addition of another reagent. SPR biosensor requires very low amounts of reagents and samples without any special preparation. It allows quick and precise determination of fibronectin concentration.

4. Conclusions

In this study, the biosensor co-operating with the SPRI technique for the selective determination of fibronectin concentration has been developed. The sensor works on the basis of a highly selective interaction between immobilized specific antibody as receptor and fibronectin in solution. SPR biosensor, as label-free detection,

has been proved to be a highly sensitive and selective method for measurement of fibronectin concentration in natural samples. This method eliminates the need for tags, dyes or specialized reagents, reduces the resources required for assay development, simplifies assay design, minimizes change of functional properties of biomolecules. The results obtained with the SPR method are similar to those obtained by ELISA. SPR biosensor can be competitive to conventional immunoassay test.

In conclusion, new SPR biosensor appears to be a good candidate for routine determination of fibronectin concentration, opening the way for applications in laboratory investigations and medical diagnostics.

Ethical approval

The informed consent was obtained by all volunteers and patients. The study had been approved by the local ethics committee and performed in accordance with ethical standards and the legislation.

Conflict of interest

The authors declare that they have no competing interests.

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References

[1] E. Yeatman, E.A. Ash, Surface plasmon microscopy, Electron. Lett. 23 (1987) 1092–1099.
[2] B. Rothenhäuslar, W. Knoll, Surface-plasmon microscopy, Nature 332 (1988) 615–617.
[3] E.A. Smith, R.M. Corn, Surface plasmon resonance imaging as a tool to monitor biomolecular interactions in an array based format, Appl. Spectrosc. 57 (11) (2003) 320–332.
[4] M. Robinson, J. Kuncova-Kallio, N. Grangvist, J.W. Sadowski, Multi-parametric surface plasmon resonance—a new technique to determine thickness and refractive index of thin and thick layers, Nanotechnology 1 (2012) 42–44.
[5] J. Zhao, X. Zhang, C.R. Yonzon, A.J. Haes, R.P. Van Duyne, Localized surface plasmon resonance biosensors, Nanomedicine 1 (2) (2006) 219–228.
[6] Y. Zeng, L. Wang, S.Y. Wu, J. He, J. Qu, X. Li, H.P. Ho, D. Gu, B.Z. Gao, Y. Shao, High-throughput imaging surface plasmon resonance biosensing based on an adaptive spectral-dip tracking scheme, Opt. Express 24 (25) (2016) 28303–28311.
[7] C. Daniel, Y. Roupioz, D. Gasparutto, T. Livache, A. Buhot, Solution-phase vs surface-phase aptamer-protein affinity from a label-free kinetic biosensor, PLoS One 8 (9) (2013), e75419, http://dx.doi.org/10.1371/journal.pone.0075419.
[8] C. Daniel, Y. Roupioz, T. Livache, A. Buhot, On the use of aptamer microarrays as a platform for the exploration of human prothrombin/thrombin conversion, Anal. Biochem. 473 (2015) 66–71.
[9] G.J. Wegner, A.W. Wark, H.J. Lee, E. Codner, T. Saeki, S. Fang, R.M. Corn, Real-time surface plasmon resonance imaging measurements for the multiplexed determination of protein adsorption/desorption kinetics and surface enzymatic reactions on peptide microarrays, Anal. Chem. 76 (19) (2004) 5677–5684.
[10] A. Shabani, M. Tabrizian, Design of a universal biointerface for sensitive, selective, and multiplex detection of biomarkers using surface plasmon resonance imaging, Analyst 138 (2013) 6052–6062.
[11] J. Ladd, A.D. Taylor, M. Piliarik, J. Homola, S. Jiang, Label-free detection of cancer biomarker candidates using surface plasmon resonance imaging, Anal. Bioanal. Chem. 393 (2009) 1157–1163.
[12] E. Matuszczak, M. Tylicka, A. Hermanowicz, W. Debek, A. Sankiewicz, E. Gorodkiewicz, Application of SPR imaging biosensor for the measurement of 20S proteasomes in blood plasma of children with thermal injury, Ann. Clin. Lab. Sci. 46 (4) (2016) 407–411.
[13] A. Sankiewicz, T. Guszcz, R. Mena-Hortelano, K. Zukowski, E. Gorodkiewicz, Podoplanin serum and urine concentration in transitional bladder cancer, Cancer Biomark. 1 (2016) 1–8.
[14] E. Gorodkiewicz, T. Guszcz, W. Roszkowska Jakimiec, R. Kozłowski, Cathepsin D serum and urine concentration in superficial and invasive transitional bladder cancer as determined by surface plasmon resonance imaging, Oncol. Lett. 8 (2014) 1323–1327.
[15] H. Chardin, K. Mercier, C. Frydman, N. Vollmer, Surface plasmon resonance imaging: a method to measure the affinity of the antibodies in allergy diagnosis, J. Immunol. Methods 405 (2014) 23–28.
[16] A. Sankiewicz, P. Laudanski, L. Romanowicz, A. Hermanowicz, W. Roszkowska-Jakimiec, W. Debek, E. Gorodkiewicz, Development of surface plasmon resonance imaging biosensors for detection of ubiquitin carboxyl-terminal hydrolase L1, Anal. Biochem. 469 (2015) 4–11.
[17] A. Sankiewicz, L. Romanowicz, P. Laudanski, B. Zelazowska-Rutkowska, B. Puzan, B. Cylwik, E. Gorodkiewicz, SPR imaging biosensor for determination of laminin-5 as a potential cancer marker in biological material, Anal. Bioanal. Chem. 408 (19) (2016) 5269–5276.
[18] W.S. To, K.S. Midwood, Plasma and cellular fibronectin: distinct and independent functions during tissue repair, Fibrogenesis Tissue Repair. 1 (2011) 4–21.
[19] P. Singh, C. Carraher, J.E. Schwarzbauer, Assembly of fibronectin extracellular matrix, Annu. Rev. Cell. Dev. Biol. 26 (2010) 397–419.
[20] R.O. Hynes, The dynamic dialogue between cells and matrices: implications of fibronectin's elasticity, Proc. Natl. Acad. Sci. U. S. A. 96 (1999) 2588–2590.
[21] L. Hirnle, I. Kaňtnik-Prastowska, Amniotic fibronectin fragmentation and expression of its domains: sialyl and fucosyl glykotopes associated with pregnancy complicated by intrauterine infection, Clin. Chem. Lab. Med. 45 (2007) 208–214.
[22] Z.Q. Xi, X. Wang, J. Luo, W. Wang, F. Xiao, D. Chen, S. Wang, M. Li, L. Wang, Fibronectin is a potential cerebrospinal fluid and serum epilepsy biomarker, Epilepsy Behav. 48 (2015) 66–69.
[23] Q. Huynh, S. Wang, E. Tafolla, S. Gansky, S. Kapila, G. Armitage, Y. Kapila, Specific fibronectin fragments as markers of periodontal disease status, J. Periodontol. 73 (2002) 1101–1110.
[24] Y. Murakami, S. Hanazawa, S. Tanaka, H. Iwahashi, S. Kitano, S. Fujisawa, Fibronectin in saliva inhibits Porphyromonas gingivalis fimbria-induced expression of inflammatory cytokine gene in mouse macrophages, FEMS Immunol, Med. Microbiol. 22 (3) (1998) 257–262.
[25] L. David, J.M. Nesland, R. Holm, M. Sobrinho Simoes, Expression of laminin, collagen IV, fibronectin and type IV collagenase in gastric carcinoma, Cancer 73 (518) (1994) 527.
[26] F. Tas, E. Bilgin, S. Karabulut, D. Tastekin, D. Duranyildiz, Levels of serum fibronectin as a biomarker in gastric cancer patients: correlation with clinical diagnosis and outcome, Mol. Clin. Oncol. 4 (4) (2016) 655–659.
[27] N. Saito, H. Nishimura, S. Kameoka, Clinical significance of fibronectin expression in colorectal cancer, Mol. Med. Rep. 1 (1) (2008) 77–81.
[28] E. Ruiz-Garcia, V. Scott, C. Machavoine, J.M. Bidart, L. Lacroix, S. Delaloge, F. Andre, Gene expression profiling identifies Fibronectin 1 and CXCL9 as candidate biomarkers for breast cancer screening, Br. J. Cancer 102 (2010) 462–648.
[29] N.K. Habash, O.F. Abdul-Rasheed, U.S. Al-Nasiri, Evaluation of serum and urinary fibronectin as a diagnostic marker of bladder cancer, Iraqi J. Med. Sci. 12 (3) (2014) 267–272.
[30] N. Mette, I.H. Ozturk, L. Colpan, Plasma fibronectin and urine glycosaminoglycan levels in rheumatic disease, Trop. J. Med. Sci. 28 (1998) 437–441.
[31] K.S. Song, H.K. Kim, W. Shim, S.H. Jee, Plasma fibronectin levels In ischemic heart disease, Atherosclerosis 154 (2001) 449–453.
[32] S.D.J. Kanters, J.D. Banga, A. Algra, R.C.J.M. Frijins, J.J. Beutler, R. Frijnheer, Plasma levels of cellular fibronectin diabetes, Diabetes Care 24 (2001) 323–327.
[33] G. Ruiz Martín, J. Prieto Prieto, J. Veiga de Cabo, L. Gomez Lus, J. Barberán, J.M. González Landa, C. Fernández, Plasma fibronectin as a marker of sepsis, Int. J. Infect. Dis. 8 (4) (2004) 236–243.
[34] S. Ali, L.A. Minhas, M. Arshad, W. Hameed, Immunohistochemical detection of fibronectin in human thymus—comparison between groups of different ages, Pak. J. Physiol. 5 (1) (2009) 45–50.
[35] X. Chang, R. Yamada, A. Suzuki, Y. Kochi, T. Sawada, K. Yamamoto, Citrullination of fibronectin in rheumatoid arthritis synovial tissue, Rheumatology (Oxford) 44 (11) (2005) 1374–1382.
[36] G. Pott, M. Meyering, B. Voss, H.E. Karges, A. Sieber, Rapid determination of fibronectin by laser nephelometry. Fibronectin concentrations is plasma in human diseases. I, J. Clin. Chem. Clin. Biochem. 18 (12) (1980) 893–895.
[37] P.T. Toy, M.E. Reid, Sample collection for determination of plasma fibronectin concentration, J. Clin. Pathol. 37 (8) (1984) 951–952.
[38] M.A. Ouaissi, L. Neyrinck, A. Capron, Development of a competitive radioimmunoassay for human plasma fibronectin, Int. Arch. Allergy Immunol. 81 (1986) 75–80.
[39] A. Hegele, A. Heidenreich, J. Kropf, R. von Knobloch, Z. Varga, R. Hofmann, P. Olbert, Plasma levels of cellular fibronectin in patients with localized and metastatic renal cell carcinoma, Tumour Biol. 25 (3) (2004) 111–116.
[40] A. Hegele, R. Hofmann, B. Kosche, J. Kropf, Evaluation of cellular fibronectin plasma levels as a useful staging tool in different stages of transitional cell carcinoma of the bladder and renal cell carcinoma, Biomark. Insights 2 (2007) 1–7.
[41] C.Y. Chen, C.C. Chang, C. Yu, C.W. Lin, Clinical application of surface plasmon resonance-based biosensors for fetal fibronectin detection, Sensors (Basel) 12 (4) (2012) 3879–3890.

[42] A.W. Peterson, M. Halter, A. Tona, K. Bhadriraju, A.L. Plant, Surface plasmon resonance imaging of cells and surface-associated fibronectin, BMC Cell Biol. 10 (2009) 1–17.
[43] E. Gorodkiewicz, M. Sienczyk, E. Regulska, R. Grzywa, E. Pietrusiewicz, A. Lesner, Z. Lukaszewski, Surface plasmon resonance imaging biosensor for cathepsin G based on a potent inhibitor: development and applications, Anal. Biochem. 423 (2012) 218–223.
[44] A. Sankiewicz, A. Tokarzewicz, E. Gorodkiewicz, Regeneration of surface plasmone resonance chips for prolonged multiple use, Bulg. Chem. Comm. 47 (2) (2015) 477–482.
[45] Q. Han, W. Kang, L. Zhang, S. Lou, Q. Zhao, Z. Liu, Increased serum fibronectin levels in patients with hemorrhagic fever with renal syndrome, Inflamm. Res. 59 (2) (2010) 135–139.
[46] A. Erturk, E. Cure, Z. Ozkurt, E. Parlak, M.C. Cure, fibronectin levels in acute and chronic viral hepatitis patients, Malays. J. Med. Sci. 21 (1) (2014) 29–36.