



An easy way to realize SPR aptasensor: A multimode plastic optical fiber platform for cancer biomarkers detection

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ABSTRACT

The introduction of new compact systems for sensitive, fast and simplified analysis is currently playing a substantial role in the development of point-of-care solutions aimed to assist both prognosis and diagnosis. Here we report a simple and low cost biosensor based on Surface Plasmon Resonance (SPR) taking advantage of a plastic optical fiber (POF) for the detection of Vascular endothelial growth factor (VEGF), selected as a circulating protein potentially associated with cancer. Our system is based onto two crucial aspects. By one hand, the functional layer which allows the transduction signal is based on DNA aptamers, short oligonucleotide sequences that bind to non-nucleic acid targets with high affinity and specificity. By the other hand, the light guiding structure is based on a POF with a planar gold layer as the sensing region, which is particularly suitable for bioreceptors implementation. The sensor revealed to be really useful in the interface characterization. The developed system is relatively easy to realize and could well address the development of a rapid, portable and low cost diagnostic platform, with a sensitivity in the nanomolar range.

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1. Introduction

Protein biomarkers are becoming increasingly significant tools from a clinical point of view. Indeed, panels of effective biomarkers have been recently proposed as prognostic factors both for easily accessible biological fluids (e.g. serum) [1] and for tissues [2] not only in oncology, but also to guide the clinical treatment of infections [3]. Once identified and validated, tests for proteins of clinical value should be incorporated into rapid, point-of-care procedures to be of real utility, for which label-free sensors are particularly suited.

The optical sensor platform here proposed is based on the Surface Plasmon Resonance (SPR) transduction, with a multimode plastic optical fiber as the light guiding structure and a planar gold layer as plasmon guide [4,5]. The planar gold layer is very helpful for immobilization of the typical molecular recognition elements used as probes for biosensor design. Moreover, the refractive index range of the liquid in contact with the platform, for which the

sensor does work, is from 1.33 to 1.42 (RIU), just the right one for measurements in aqueous medium, so it is particularly suited for determination in biological fluids.

When a bioreceptor is immobilized on the surface of metal (gold), the analyte present in a liquid sample is selectively recognized and captured, thus producing a local increase in the refractive index at the gold surface. This change is detected by SPR, as a suitable optical interrogation [6–8].

For low-cost sensing systems, POFs are especially advantageous due to their easy manipulation, excellent flexibility, great numerical aperture, large diameter, and the fact that plastic is able to withstand smaller bend radii than glass.

The optical sensor platform here proposed, based on POF, has already been tested in bio-application [9] and in low molecular weight substances detection [10–12], using different receptors. For example, the effectiveness of molecularly imprinted polymers as receptors in the case of low molecular weight substances [10,11] and of antibodies in the case of proteins [9] has been demonstrated. We propose here the application of the same kind of optical platform in the detection of cancer biomarkers, using aptamers as specific receptors.

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As a proof of principle, we selected the Vascular endothelial growth factor (VEGF) as a circulating protein potentially associated with cancer.

This protein has a crucial role in the angiogenesis event. Besides a normal protein activity during embryonic organ growth and development, or during reproduction or physiological repair in adults [13], a significant presence in pathological conditions is observed [14]. A clinical correlation between cancer patients and VEGF levels has been recently proposed [13], suggesting that VEGF could be a significant cancer biomarker. The bioreceptor considered in the present investigation for the recognition of VEGF is based on DNA-aptamers, which are short single-stranded DNA or RNA molecules, having high selectivity and affinity toward their targets. Aptamers present many advantages over antibodies, such as high reproducibility in target recognition, easiness in being chemically modified and high stability even in non-physiological conditions [15]. Thanks to these features, they are widely applied in the biosensor field [16]. Different detection methods are utilized, based on Surface Plasmon Resonance [17,18], electrochemistry [19,20], optics (fluorescence) [21,22], chemiluminescence [23] or even label-free detection [24]. Label-free detection is particularly relevant for fast, on-site, low cost tests.

In literature some examples of sensors combining optical fiber detection and aptamers are reported [25,26], based on a standard optical fiber, and with an experimental set up much more convenient than the prism based Kretschmann configuration. Here we present the first SPR aptasensor built in a POF for protein biomarkers label-free detection. This optical platform based on an optimal design of D-type POFs for best sensitivity of SPR sensors is low cost, small size and easy to realize. As a proof of concept, we have immobilized on the gold surface an aptamer as the bioreceptor layer for VEGF detection. The functionalization process is characterized from an optical and a chemical point of view, using fluorescence measurements and X-ray Photoelectron Spectroscopy (XPS) to analyze and quantify the aptamer molecules at the gold surface. Finally, the detection of VEGF is reported in a nanomolar

range, strongly suggesting that sensors of this kind can be used in the protein biomarker recognition.

2. Experimental section

2.1. Materials

β -Mercaptoethanol (MP-ET) and all powders for buffer solutions were purchased from Sigma-Aldrich s.r.l. (Milan, Italy). DNA-aptamer sequence specific for VEGF (5'-HO-(CH₂)₃-S-S-(CH₂)₃-CC CGT CTT CCA GAC AAG AGT GCA GGG-3') is HPLC purified and was purchased from IDT Integrated DNA technologies (Leuven, Belgium). The fluorescein labeled sequence (5'-HO-(CH₂)₃-S-S-(CH₂)₃-CC CGT CTT CCA GAC AAG AGT GCA GGG-(CH₂)₃-fluorescein-3') was purchased from the same seller. Purified VEGF₁₆₅ (0.3 mg/ml) and Bovine serum albumin (BSA) were purchased from Sigma-Aldrich s.r.l. (Milan, Italy).

Gold flat substrates are utilized to characterize the functionalization process.

2.2. Optical sensor fabrication and experimental setup

A schematic representation of the POF-SPR is reported in Fig.1(a). The plastic optical fiber has a PMMA core of 980 μ m and a fluorinated polymer cladding of 20 μ m. The experimental results indicate that the configuration with a fiber of 1000 μ m in diameter exhibits good performance in terms of sensitivity and resolution but less good in terms of signal to noise ratio (SNR) [5]. The fabricated optical sensor platform was realized as previously described [4,5], removing the cladding of the plastic optical fiber along half the circumference, spin coating on the exposed core a buffer of Microposit S1813 photoresist, and finally sputtering a thin gold film using a sputtering machine. The realized sensing region was about 10 mm in length. The gold film was 60 nm thick and presented a good adhesion to the photoresist (the thickness of photoresist buffer layer was about

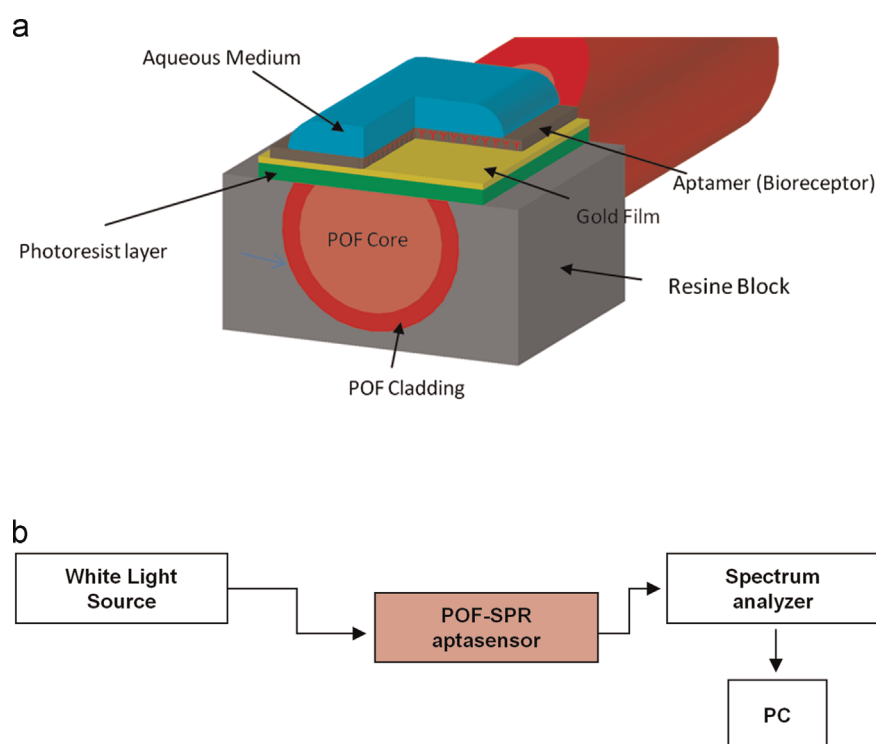


Fig. 1. (a) Scheme of the POF-SPR aptasensor, with a cross-section of the sensor system. (b) Experimental setup.

1.5 μm). This planar gold layer can be employed for depositing the receptor layer (aptamer).

The experimental setup was arranged to measure the light spectrum transmitted through the POF aptasensor and was characterized by a halogen lamp, illuminating the optical aptasensor system and a spectrum analyzer, see Fig. 1(b). The employed halogen lamp exhibits a wavelength emission range from 360 nm to 1700 nm, while the spectrum analyzer detection range was from about 330 nm to 1100 nm. An Ocean Optics USB2000+VIS-NIR spectrometer has been employed. The spectral resolution of the spectrometer was 1.5 nm (FWHM). The spectrometer is finally connected to a computer. The SPR curves along with data values were displayed online on the computer screen and saved with the help of advanced software provided by Ocean Optics. The processing of experimental results was carried out by Matlab software. SPR transmission spectra were obtained by normalizing to the spectrum achieved with air as the surrounding medium. A minimum in the transmission spectrum corresponds to the gold surface plasmon resonance.

2.3. Gold layer preparation and functionalization

The gold film was firstly cleaned with an argon plasma, applying 6.8 W of power to the RF coil for one minute to remove organic contaminants. Before the functionalization process, aptamers were subjected to thermal shock (95 °C for 1 min, ice for 10 min) in order to unfold the sequence strands and make the dithiol groups available for the immobilization reaction. The functionalization procedure was then accomplished by immersion into a 1 μM aptamer solution in 1 M potassium phosphate buffer pH 7 for one hour. Two different kinds of aptasensor have been realized, one derivatized with aptamer only (Apt) and one with aptamer and passivating agent (mercaptoethanol) (Apt-MPET). To obtain Apt-MPET, a passivation step in 1 mM mercaptoethanol solution in the same buffer for 30 min was performed after functionalization with the aptamer. After washing in buffer and finally in ultrapure MilliQ water, the aptasensors were ready to use.

The VEGF detection and BSA control was done in 20 mM Tris-HCl buffer pH 7.4.

All sensing measurements were performed in a laboratory room with a temperature control set at 23 °C.

2.4. Surface characterization

The functionalization process on flat gold substrate was characterized using XPS and fluorescence measurements. The quantification of the aptamers immobilized on the gold surface was performed using a fluorescent derivative sequence, that is the same of the specific one, with a fluorescein molecule at the 3' end.

The fluorescence signal was monitored in two different ways. The aptamer distribution and uniformity on the gold surface was analyzed directly in the solid phase using a fluorescence microscope (Leica DMLA; Leica Microsystems, Germany) equipped with a mercury lamp and the fluorescence filter L5 (Leica Microsystems, Germany). All samples were observed with a 40 $\times$ magnification objective and measured with a cooled CCD camera (DFC 420C, Leica Microsystems, Germany). The signal was analyzed using the ImageJ software [27]. The aptamer molecules immobilized on the surface were quantified by measuring the fluorescence signal in solution, after an overnight detachment in 1 mM mercaptoethanol. The signal was obtained with a FluoroMax[®]-4 spectrofluorometer (Horiba Scientific, Japan), exciting at 480 nm and acquiring the signal between 496 and 660 nm. A calibration curve of the fluorescent probe in solution at the same conditions was prepared.

XPS measurements were performed using a Kratos Axis Ultra DLD instrument equipped with a hemispherical analyzer and a monochromatic Al K α (1486.6 eV) X-ray source, in transmission mode. The emission angle between the analyzer axis and the sample surface was 90° and 30°, corresponding to a sampling depth of approximately 10 nm and 2–3 nm, respectively [28]. For each sample O 1s, C 1s, N 1s, P 2p, S 2p and Au 4f core lines were recorded. The quantification, reported as relative elemental percentage, was done by using the integrated area of the fitted core lines, after Shirley background subtraction, and by correcting for the atomic sensitivity factors. This procedure gives a semi-quantitative analysis, which however is useful for the chemical characterization of the surface at different modification steps.

3. Results and discussion

The performances of the sensing device are strictly related to the quality of the sensor's interface. Therefore, we selected a gold layer deposited on silicon substrate as representative of sensor's surface, and characterized the gold layer and the interaction between the gold layer and the aptamers molecules from a chemical point of view using XPS technique. Monitoring and quantifying the aptamer density was performed through fluorescence measurements.

3.1. Chemical surface characterization by XPS

The chemical composition of the gold surface was analyzed immediately after the cleaning procedure, which consisted in an Argon plasma treatment. The atomic percentage of the principal elements recorded is reported in Table 1. Evidently the cleaning procedure is not able to completely remove the organic contaminations (sample "A" in Table 1). The high carbon content (especially at 30° take-off angle, Table 1b) suggests an hydrocarbon contamination

Table 1
Elemental composition (atomic percentage) determined by XPS analysis at 90° and 30° take-off angles, (a) and (b) respectively, on gold surface after different treatments. The error does not exceed the 1–2% of the reported values.

Sample	Surface treatment	O1s (%)	N1s (%)	C1s (%)	S2p (%)	P2p (%)	Au4f (%)
(a)							
A	Plasma-treated	6.9	–	17.4	2.6	–	73.1
B	Aptamer only (1 h)	20.7	9.7	31.2	0.7	2.7	35.0
C	Aptamer only (24 h)	20.4	9.7	32.9	1.7	2.5	32.8
D	Aptamer (1 h)+MPET	20.3	8.4	29.6	2.0	2.6	37.1
(b)							
A	Plasma-treated	8.0	–	28.3	3.3	–	60.4
B	Aptamer only (1 h)	22	11.9	42.2	1.0	3.8	19.1
C	Aptamer only (24 h)	29.9	11.2	42.3	1.8	3.5	19.3
D	Aptamer (1 h)+MPET	21.8	8.9	40.4	2.2	3.5	23.2

coming both from the fabrication process and from the environment. The gold content however is sufficient to ensure a good aptamer bonding. The increase in the carbon and oxygen signals recorded on the aptamer incubated sample (sample “B” in Table 1) confirms the immobilization of the sequence as suggested also by the appearance of phosphorus and nitrogen signals related to the DNA chain. As expected, the aptamer fingerprints (N1s and P2p) increase at increasing superficial recording angle (Table 1b). No significant difference is observed between one or 24 h incubated sample (sample B and C respectively, Table 1), suggesting that a short incubation time is sufficient to obtain the coverage of gold substrate. Finally, the surface passivation by mercaptoethanol (MP-ET) for 30 min, as reported in the experimental part (sample “D” in Table 1), partially displaces the aptamers from the surface, as suggested by the decrease of nitrogen and carbon content and by the increase in the gold and sulfur signals. The chemical composition is in agreement with literature [29,30]. The passivating agent has an important role in the aptamer orientation as discussed in the following paragraphs.

3.2. Aptamer layer characterization by fluorescence and by SPR

The density of the aptamer layer was investigated using a fluorescent derivative aptamer sequence immobilized on a model gold flat surface treated exactly as the gold layer on the sensor. The fluorescence microscopy analysis of the gold surface at different incubation time is reported in Fig. 2. After an initial absorption in an irregular way (Fig. 2A), the surface was covered by a uniform layer for a 60 min incubation (Fig. 2C). This heterogeneity in the fluorescence could be related to the DNA packing density. More brilliant areas are related to “floating monolayer”, i.e. sequences far from the gold surface, as suggested by Murphy et al. [31]. The higher fluorescence signal is recorded on the surface incubated for 150 min (Fig. 2D), while a decrease in the signal is observed after an overnight incubation (Fig. 2E). This behavior could be due to different reasons. It could be guessed that a rearrangement of the adsorbed aptamer takes place for long incubation times, leading to an interaction between nucleotides and gold surface resulting in fluorescence quenching [32,33]. This hypothesis is supported by the XPS analysis (Table 1), according to which the density of the aptamers on the gold surface at one hour incubation is rather similar to that with overnight incubation.

The density of the aptamer immobilized at the gold surface in function of the incubation time was determined by stripping out the molecules from the gold surface by an overnight incubation in mercaptoethanol solution, and measuring the fluorescence signal of the detached aptamers in the solution phase. A calibration curve previously worked out was used for quantification, as described in Section 2.3. Incubating the aptamer solution for 10 min, a density of 3×10^{12} molecules/cm² is obtained. The density was stabilized at 7×10^{12} molecules/cm² after one hour incubation. These values are in agreement with those by Steel et al. [34], who reported 1×10^{13} molecules/cm², finding a similar behavior as function of time. Moreover, the density of the aptamers on the gold surface at one hour with respect to an overnight incubation is rather similar, as confirmed by XPS analysis (Table 1).

The immobilization of the aptamer sequence on the sensor surface is also confirmed by SPR measurements using directly the optical platform proposed. Fig. 3 presents the SPR transmission spectra, normalized to the spectrum obtained in air as the surrounding medium, for the sensor in Tris–HCl buffer, before and after the functionalization with aptamers. A red-shift in the transmission spectrum was indeed detected when aptamers were immobilized on the sensor surface (see Fig. 3a) as in the case of Apt. The shift due to the aptamer immobilization is 14.8 ± 3.9 nm.

In the case of Apt–MPET, i.e. when the functionalization process is completed with the subsequent binding of passivating agent (mercaptoethanol), a much larger red-shift of 45 ± 3.5 nm is observed (see Fig. 3b). Evidently a local increase in the refractive index is produced at the metal surface, which is ascribed to the aptamer molecules blocked on the gold surface. In fact the refractive index increase gives rise to an increase in the propagation constant of Surface Plasmon Wave (SPW) propagating along the metal surface, and the resonance wavelength shifts to longer wavelengths (red shift) [4,9]. Since the red shift is much higher for Apt–MPET, the refractive index variation appears to be higher for the binding of mercapto-ethanol than for the aptamer.

3.3. Effect of the sample solution composition on the resonance wavelength

When a new sensor is contacted with the buffer used for measurements, 20 mM Tris–HCl, pH 7.4, not any variation of the resonance wavelength on time is observed in the case of Apt–MPET, in which the functionalization process is followed by passivation with mercaptoethanol. On the contrary a very peculiar behavior was observed in function of time in the case of Apt, obtained by functionalizing the gold surface with aptamers only. The transmission spectrum in buffer solution, not containing the protein of interest, showed a remarkable blue-shift (Fig. 4) of the resonance wavelength on time. Even after one hour incubation a stable signal was not obtained. It is relevant to notice that this effect is completely reversible, i.e., if the surface was made completely dry, and then re-hydrated in buffer solution, the starting resonance wavelength value was completely restored, and the same blue-shift effect with time was observed again.

The observed behavior could be tentatively explained considering that in the measurement buffer the DNA sequence adsorbed could undergo a rearrangement, drifting the aptamer bases away from the gold surface, and allowing some aqueous solution to reach the gold interface, in this way producing a decrease of the refractive index of the layer in contact with the metal surface. Alternatively, it could be thought that the aptamer molecules are completely stripped away in the buffer solution, which has a composition different from that of the buffer used for the functionalization, but this seems not to be the case, since the observed effect is reversible, as mentioned above. On the contrary, in the case of Apt–MPET, the passivating molecules of mercaptoethanol might cause the aptamers to stand up from the gold surface, keeping the nucleotide molecules partially desorbed from the gold surface at all the conditions, and at the same time they could prevent the buffer solution to reach the gold interface.

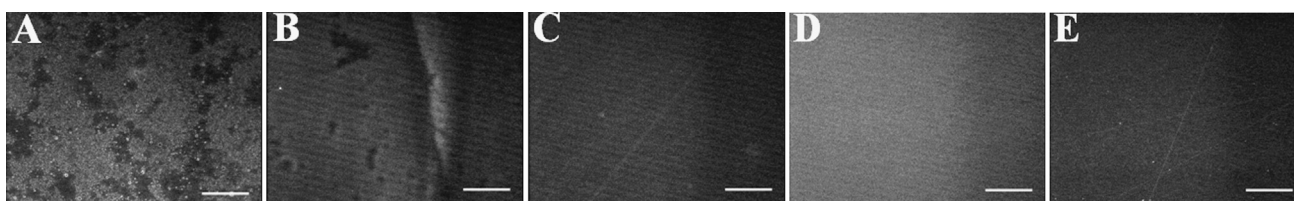


Fig. 2. Fluorescence microscopy images of fluorescein-tagged aptamer on gold at different incubation times: 10 min (A), 30 min (B), 60 min (C), 150 min (D) and after overnight incubation (E). The scale bar is 50 μ m in all images.

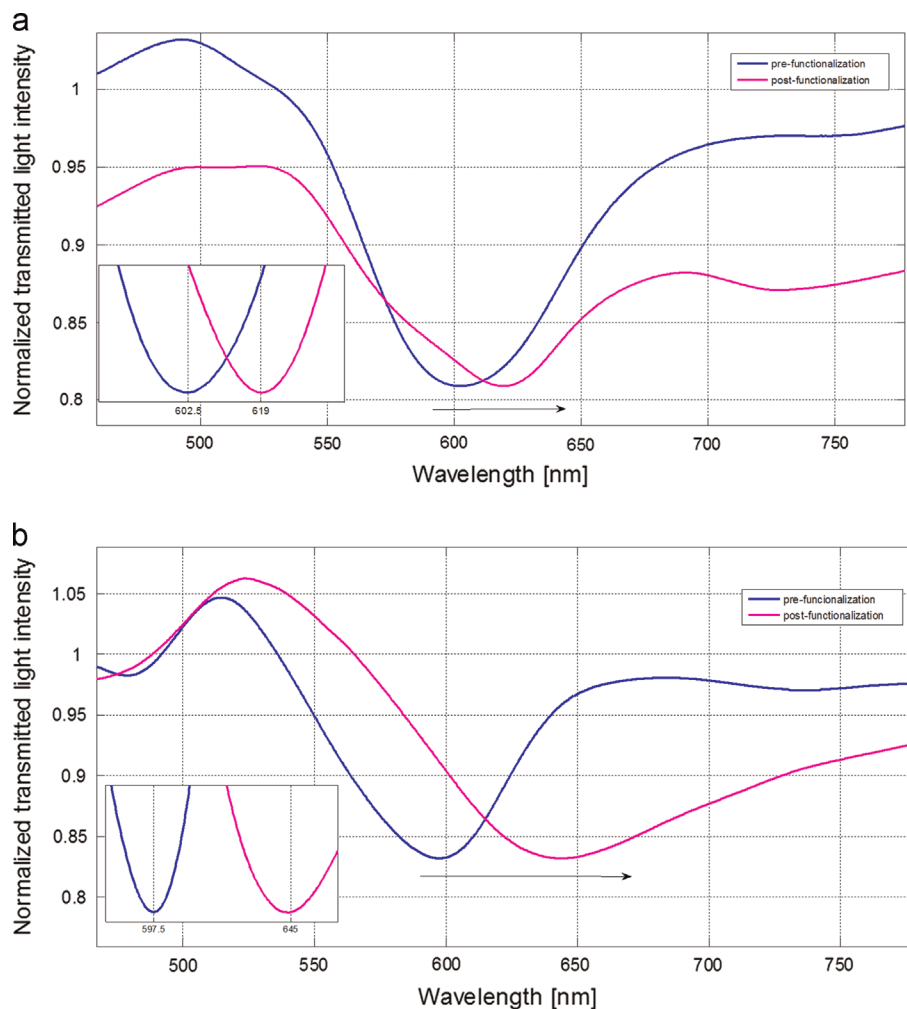


Fig. 3. SPR transmission spectra obtained before (line with minimum transmittance at lower wavelength) and after (line with minimum transmittance at higher wavelength) the functionalization with only aptamers (Apt) (a) or with aptamers followed by passivation (Apt-MPET) (b). Measurements have been made by dropping 100 μ l of 20 mM Tris-HCl pH 7.4 buffer over the sensing surface.

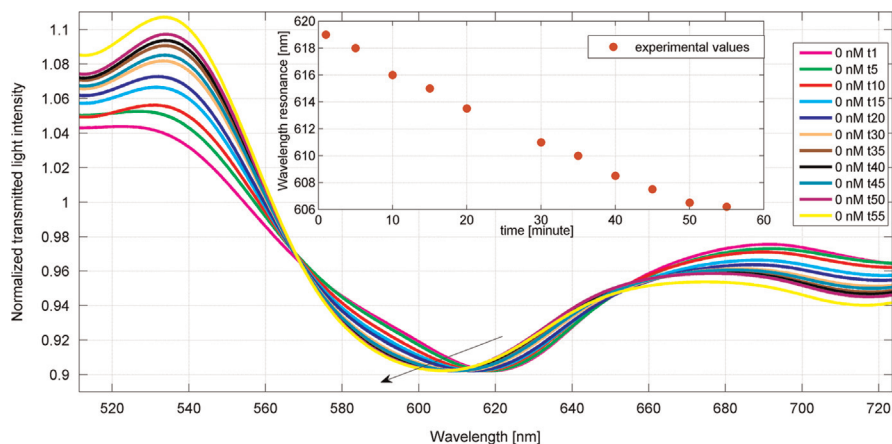


Fig. 4. SPR transmission spectra obtained in blank buffer solution (0 nM VEGF) immediately after functionalization with aptamer only (Apt) (pink line) during the time up to one hour (yellow line). Time increases as indicated by the arrow. Inset: resonance wavelength as function of time. (For interpretation of the references to color in this figure legend, the reader is referred to the web version of this article).

This interpretation of the optical behavior of Apt and Apt-MPET is in agreement with the literature [34], where it is reported that sequences longer than 24 nucleotides behave as flexible, coil-like polymer chains that adsorb side by side on the surface. This is also confirmed by the fluorescence measurements, as seen in Fig. 2. It could be surprising that a chemical rearrangement involving probably

not more than 10 nm at the gold surface is able to give such high SPR signal variation, nevertheless it must be recalled that the electric field of evanescent wave, on which the SPR relies, decreases in an exponential way, so the strongest interaction occurs exactly very near to the interface. The comparison of the behavior on the time of Apt (no passivation) with Apt-MPET (passivation with mercaptoethanol)

leads to the conclusion that Apt-MPET must be preferred for the stability of the signal.

3.4. Sensing measurements

When receptors are used for bio/chemical detection, the film on the surface of metal selectively recognizes and captures the analyte present in a liquid sample so producing a local variation of the refractive index at the metal surface, as seen in Fig. 5. The value of the refractive index increment depends on the structure of the analyte molecules. The refractive index change can be accurately measured, as a shift of the resonance wavelength, since the propagation constant of the SPW is proportional to the refractive index change and the depth of the area within which the change occurs. The sensitivity can be defined as the shift in resonance wavelength per unit change in analyte concentration (nm/M).

In this work, the detection of VEGF is investigated, as a proof of concept, for the protein biomarkers detection by SPR on POF with aptamers as receptors.

The sensitivity of the SPR aptasensor system is strongly influenced by the structure of the interface layer. When the aptamer only is immobilized on the surface (Apt), high protein concentrations are required to observe a red-shift (low sensitivity). A 50 nM of VEGF caused a shift of about 6 nm, as reported in Fig. 5. The kinetic of binding is completed in 18 min. Fig. 6 shows the resonance wavelength as function of time for this sensor.

When the passivating molecules are immobilized on the sensor surface after the aptamer (Apt-MPET), a more sensitive interface is obtained. For example, a 1 nM VEGF solution caused a red-shift of about 1 nm (see Fig. 7), so with a sensitivity about 8.3 times better than that of the non-passivated sensor

In both sensors the standardization curve is not a straight line, probably due to the saturation of the limited number of receptor sites at the gold/dielectric interface. The experimental points can be better fitted by the Langmuir adsorption model [35], assuming that the adsorption on the sites in the active layer takes place according to the following equilibrium:



It is possible to evaluate the affinity constant ($K_f = [\bar{A}P]/[A][\bar{P}]$) and the product of the proportionality constant (k) of the signal and the concentration by the concentration of the receptor sites (c_{site}). According to this model the analytical response ($\Delta\lambda$) depends on the analyte concentration c as here reported:

$$\Delta\lambda = \frac{kc_{\text{site}}(K_f c)}{1 + (K_f c)} \quad (2)$$

The values obtained for the parameters K_f and kc_{site} are shown in Table 2. $kc_{\text{site}} K_f$ is the slope of the straight line obtained at very low $K_f c$, i.e. for c much lower than K_f , (the slope corresponds to the sensitivity at low concentration) and kc_{site} is the limiting value of

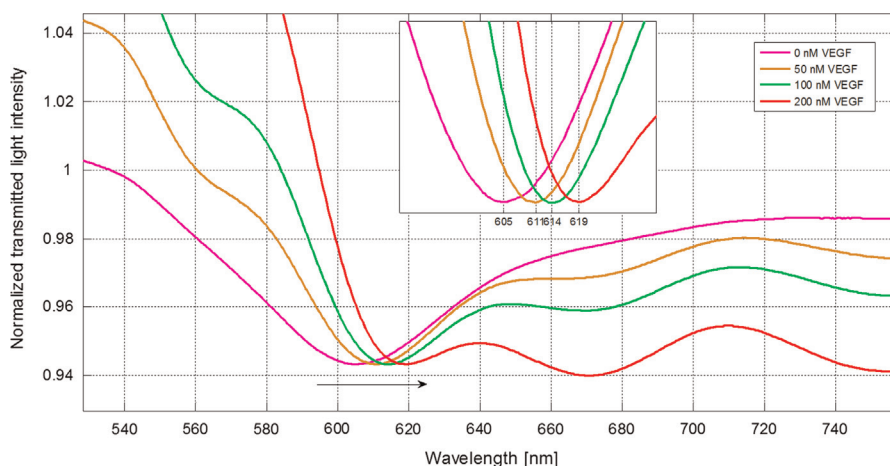


Fig. 5. SPR transmission spectra obtained incubating different amount of VEGF (0–200 nM) on sensor functionalized with aptamer only (Apt). VEGF concentration increases as indicated by the arrow. Inset: zoom of the resonance wavelengths region.

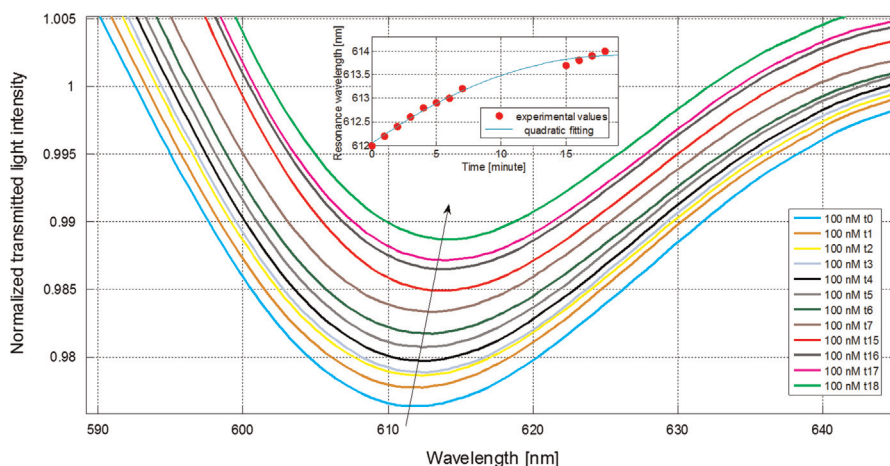


Fig. 6. SPR transmission spectra as a function of time, at sensor functionalized with aptamer only (Apt), in 100 nM of VEGF. Times increases as indicated by the arrow. Inset: Fitting of the resonance wavelength as a function of time.

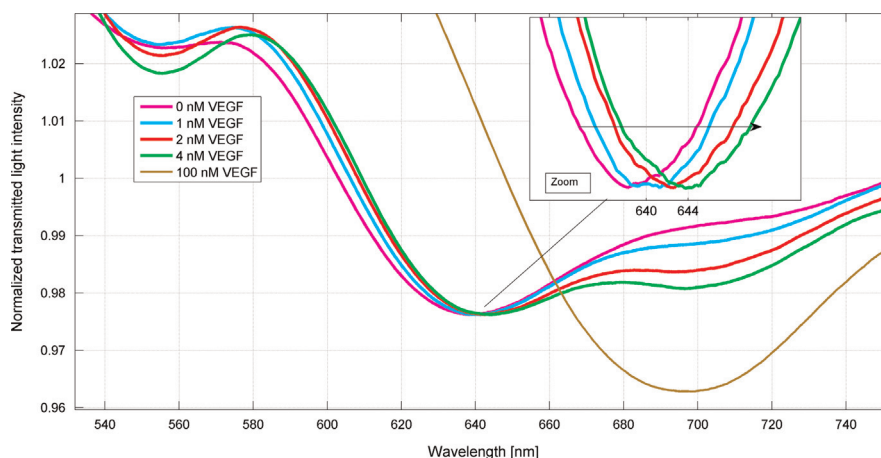


Fig. 7. SPR transmission spectra obtained incubating different amount of VEGF (0–100 nM) on sensor functionalized with aptamers followed by passivation (Apt–MPET). VEGF concentration increases as indicated by the arrow. Inset: zoom of the resonance wavelengths region.

Table 2

Parameters of the Langmuir equation for two different aptasensors with aptamer only (Apt) and with aptamer and mercaptoethanol (Apt–MPET) and kinetical parameters for the VEGF determination. k is the proportionality constant between $\Delta\lambda$ observed and the concentration of the protein in the sample solution.

Aptasensor	Equilibrium parameters			Kinetical parameters		
	K_f (M^{-1})	$kc_{site}K_f$	kc_{site} (nm)	$k_{kinetical}$ ($M^{-1}s^{-1}$)	$K'c_{site}$	
Apt	$7.5(3) \times 10^6$	$1.7(3) \times 10^8$	22.07	1.9×10^4	2.1	
Apt–MPET	$2.1(9) \times 10^7$	$1.3(4) \times 10^9$	74.4	8.0×10^5	1.7	

the analytical signal at very high concentration of the analyte. The limit of detection of Apt, the sensor not passivated with mercaptoethanol, is approximately 3 nM (obtained from 2x standard deviation of the blank), while that of the passivated sensor (Apt–MPET) is 0.8 nM. For comparison the experimental values obtained with the two considered aptasensors, are reported in Fig. 8, in which the fitted curves, calculated with the parameters reported in Table 2, are shown too.

The inset of Fig. 6 shows the shift of the resonance wavelength as a function of time in the case of the sensor with the aptamer only (Apt). The continuous curve has been calculated by considering a pseudo-first order kinetics with respect to the concentration of the aptamer combination sites, using the Eq. (3), where $K'c_{site}$ represents the proportionality between the wavelength shift ($\Delta\lambda$) and the complex aptamer–protein concentration, while $k_{kinetical}$ is the kinetic constant. The parameters obtained fitting the experimental data are reported in Table 2. Similar results have been obtained in the case of the sensor with aptamer and mercaptoethanol (Apt–MPET)

$$\Delta\lambda = K'c_{site} - K'c_{site} * e^{-k_{kinetical} * t} \quad (3)$$

It is particularly interesting that the $k_{kinetical}$ constant is much higher in the case of Apt–MPET. This is possibly due to the fact that the interaction sites in the aptamers are more easily accessible in the surface passivated sensor.

Comparing the dissociation constant for the aptamer sequence reported by Potty et al. [36] with the values obtained fitting the Eq. (2), a difference of about two orders of magnitude is seen. This could be explained assuming that the direct immobilization of the aptamer sequence on gold substrate entails a partial loss of the molecule affinity for the target. Examples to solve this problem are reported in literature [25,26,37], where an intermediate layer mainly based on PEG moieties is first immobilized on gold. This layer reduces non-specific adsorption due to both its high hydrophilic character and to the steric repulsion resulting from the compression of PEG chains.

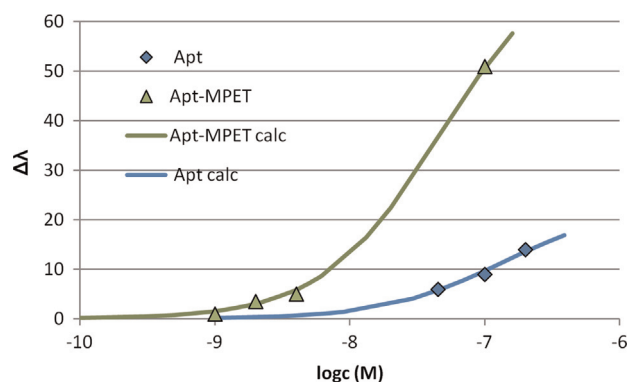


Fig. 8. Experimental values: resonance wavelength variation against the logarithm of VEGF concentration. Fitted curve: Langmuir equation in the usual lin-log form, for both aptasensors Apt and Apt–MPET.

Therefore, although a passivation process with mercaptoethanol increases the sensitivity of our sensor, better conditions than those here used can be implemented in order to preserve the aptamer functionality and ensure the best protein recognition.

A solution without VEGF, but containing BSA at high concentration (up to 100 nM) in the same Tris buffer solution has been tested as a negative control on Apt–MPET sensor. Not any SPR shift has been observed with respect to the pure buffer showing the good selectivity of the proposed device, due to the high selectivity of the aptamer, and to the successful passivation procedure.

4. Conclusions

Here we presented a low cost biosensor based on SPR optical detection with an integrated POF waveguide. The sensor is functionalized using aptamers as biorecognition agents. The proof of concept of its employment in clinical diagnostic is demonstrated in the case of VEGF, a circulating cancer biomarker. The aptamer-based interface has been monitored using fluorescence measurements in solid and liquid phase, XPS analysis, and SPR measurements by the here proposed setup based on POF too. By the passivation with mercaptoethanol an improved sensor has been obtained, with increased sensitivity and stability of the signal.

Once obtained the best aptamer layer, the detection of VEGF has been demonstrated in the nanomolar range. The experimental results indicate that this new aptasensor based on SPR on POF exhibits good performances when the passivating molecules are

immobilized on the sensor surface after the aptamer immobilization. The passivation process has however to be further optimized in order to improve the aptamer affinity and the kinetical characteristics.

Improvements in the sensor sensitivity could be obtained by modifying the geometry of the optical platform, or by improving the fluidic chamber and the environment control in order to reduce the noise level.

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