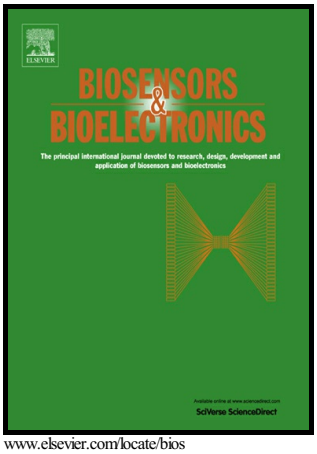

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Cancer biomarker sensing using packaged plasmonic optical fiber gratings: towards *in vivo* diagnosis

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Abstract

This work presents the development of an innovative plasmonic optical fiber (OF) immunosensor for the detection of cytokeratin 17 (CK17), a biomarker of interest for lung cancer diagnosis. The development of this sensing platform is such that it can be assessed in non-liquid environments, demonstrating that a surface plasmon resonance (SPR) can be excited in this case. For this purpose, detections have been first carried out on CK17 encapsulated in gel matrix in the aim of mimicking tissue samples. Gold-coated OF immunosensors were embedded in a specifically designed packaging providing enough stiffness to penetrate into soft matters. Resulting reflected spectra have revealed, for the first time, the presence of a stable SPR signal recorded in soft matters. Experiments conducted to detect CK17 trapped in a porous polyacrylamide gel matrix have highlighted the specific and selective biosensor response towards the target protein. Finally, the packaged OF immunosensor has been validated by a preliminary test on human lung biopsy, which has confirmed the *ex-vivo* CK17 detection. Consequently, this work represents an important milestone towards the detection of biomarkers in tissues, which is still a clinical challenge for minimally-invasive *in vivo* medical diagnosis.

Keywords:

Fiber optic biosensor

Tilted fiber Bragg grating (TFBG)

Surface plasmon resonance (SPR)

Cytokeratin 17 (CK17) detection

Biomarkers detection in soft matters

Catheter and mechanical packaging

1. Introduction

The presence of tumoral tissue in patients remains the target of most diagnoses. Unfortunately, at early stages, cancer is not easily visible, which explains some delayed findings, with severe or fatal consequences. Furthermore, to avoid misdiagnosis, numerous biopsies are carried out in order to confirm/infirm the clinical verdict, which results in a large amount of unnecessary surgery acts causing pain and discomfort for patients. It is well known that survival for patients with cancer is strongly correlated to an early stage diagnosis. There is therefore a need for new tools to facilitate and to specially accelerate the clinical diagnosis *in vivo*.

For decades, the detection of biomarkers has been a major trend (Henry and Hayez, 2012). They have indeed been extensively studied and proven to play key roles in numerous pathogenic processes (Burke, 2016). Research on biomarkers is conducted routinely for cancer diagnosis, since their presence or absence allows indicating the type of cancer. The identification of biomarkers is currently performed on tissues, which requires to take biopsies. To the aim of avoiding surgical acts, the idea to detect biomarkers *in vivo* has emerged, which resulted on the elaboration of dedicated biosensors.

Biosensors were mainly developed in the aim to detect biomarkers of interest (Wu and Qu, 2015; Justino et al., 2016). The label free and real time detection proposed by this technology remains an important asset compared to classical techniques. The miniaturized size coupled to a good sensitivity are also important for biomarker detection. For many years, numerous kinds of biosensors have thus been elaborated, differentiated by their targets (proteins, cells) or their transduction modes including electrochemical or optical techniques amongst others (Bohunicky and Mousa, 2010). Nevertheless, few of them have exceeded the laboratory stage, also because of the complexity and the difficulty to access real samples. Only biosensors based on the detection of biomarkers present in biological fluids such as blood or urine can easily be tested on collected samples (Khurshid et al., 2016; Mishra et al., 2016), but they are unfortunately not usable for *in vivo* detections.

The access to biomarkers present in tumor tissues - which could help to determine the nature of the cancer - is a milestone to obtain a reliable diagnosis. However, the detection of biomarkers in *in vivo* conditions remains a big challenge, notably due to their location. Techniques for measurement in soft matters have to be consequently developed in order to closely mimic the real experimental conditions. The detection in soft matters implies additional constraints in the elaboration of the biosensor. It has to be specifically designed in order to penetrate soft matters in the least invasive manner, while maintaining a high sensitivity and above all a rapid acquisition time.

Here we report for the first time the feasibility of the specific measurement of a biomarker encapsulated in gel matrix using a surface plasmon resonance (SPR) optical fiber sensor (Sharma et al., 2007). To the best of our knowledge, no study has been reported on

the use of SPR biosensors for the detection of biomarkers in soft matter. A validation conducted in a fresh biopsied human tissue further demonstrates the potential of the technique.

SPR is a widespread technology among optical biosensors (Singh, 2016). A large number of studies that focus on the optical detection of biomarkers have been reported in the literature (Yoo and Lee, 2016). Nevertheless, none has been conducted on soft matter, principally due to the commonly used Kretschmann prism configuration. However, for a few years now, a class of SPR biosensor based on optical fiber (OF) has emerged (Sharma et al., 2007). In contrast with the traditional flat substrate, the cylinder shape and the micrometer size of OF as well as its flexibility represent keys assets that can be used for measurements in soft matters. Moreover, optical fibers can be used as dip probe, which helps to easily provide access to various samples.

SPR excitation in OF can be performed by two main techniques (Barnes et al., 2003, Caucheteur et al., 2015). The first configuration is made of unclad glass fibers covered with a nanometric layer of gold (Lu et al., 2016; Shi et al., 2015). The second technique used in this study is based on the photo-inscription of tilted fiber Bragg gratings (TFBGs) in the core of a gold-coated glass optical fiber (Albert et al., 2012). SPR-TFBGs firstly appeared in 2007 with the work conducted in the team of J. Albert (Shevchenko and Albert, 2007) and have since demonstrated a very good sensitivity towards various targets including proteins (Guo et al., 2016; Voisin et al., 2014) and eukaryotic cells (Malachovskà et al., 2015; Guo et al., 2014). We have demonstrated in our previous work a limit of detection around 1 pM for proteins (Ribaut et al., 2016). This high sensitivity is especially due to the detection in the near-infrared wavelengths (1500-1600 nm). Although gold-coated TFBGs gather many advantages such as easy handling, their direct use on soft matters is compromised due to the high flexibility and fragility of silica. Polymer OFs (POFs) that bring the advantage of being biocompatible have started to emerge but their current sensitivity reaches lower performance than glass optical fiber (Amin et al., 2012; Cennamo et al., 2015; Hu et al., 2014). Consequently, to penetrate into soft tissues with our optical fiber optrodes, we have developed a 1.2 mm Ø biocompatible packaging specially designed to embed an optical fiber (Ø = 125 µm diameter) and protect it from possible damage (Fig. 1.B).

In the course of our reflection regarding the choice of the soft matter that will be used for extensive experiments, we faced the challenge to find a tissue-equivalent material. Hydrogels made of Poly(vinyl alcohol) are known to substitute tissue due to their similar biomechanical characterization and morphological structure (Jiang et al., 2014). However, the seven freeze/thaw cycles required for their synthesis make them not compatible with the use of biomolecules. After careful considerations, polyacrylamide gels were selected. They are often used for the separation of protein mixtures. We have thus built a novel biosensor merging robustness and sensitivity for the detection of biomarkers in polyacrylamide gels. To demonstrate the efficiency of our innovative biosensor, the SPR-

TFBG device was tested on proteins of interest in the lung cancer diagnosis, namely Cytokeratin 17 protein (CK17) (Chen et al., 2011) that have been encapsulated in an acrylamide gel matrix. Finally, to validate the feasibility to use this device in real conditions, detections have been carried out on a freshly collected human lung biopsy.

2. Materials and methods

2.1 Materials

Fetal Bovine Serum (FBS), N-Hydroxysuccinimide (NHS) ; N-(3-Dimethylaminopropyl)-N'-Ethylcarbodiimide Hydrochloride (EDC), Phosphate Buffer Saline (PBS), Ammonium Persulfate (APS) were purchased from Sigma-Aldrich. Anti-Cytokeratin 17 antibody (AbCK17) and Cytokeratin 17 (CK17) human protein came from Abcam. Bovine Serum Albumin (BSA) was from Acros. DithiolalkanearomaticPEG6-COOH (S_2-PEG_6-COOH) came from SensoPath technology. 30% Acrylamide/bis-acrylamide (29:1) 3,3C% crosslinked solution and Tetramethylenediamine (TEMED) were from Bio-Rad. Buffers were prepared in milliQ water and polyacrylamide gels in pH 7.4 PBS buffer. POM packaging came from Ticona Engineering Polymers. The silver conductive paint was purchased from CMR-Direct Company.

2.2 Biosensor elaboration

The scheme displayed in Fig. S1. Summarize the different steps required for the elaboration of the biosensor.

2.2.1 TFBG photo-inscription

The photo-inscription of TFBG has already been described in our previous work (Caucheteur and Mégret, 2005). Briefly, a telecommunication-grade single-mode optical fiber (Corning SMF-28, 125 µm uncoated silica fiber diameter) was used to prepare each sensor. TFBGs were inscribed in the fiber core, previously hydrogenated, with an external tilt angle of 7 °. 1 cm long TFBGs were photo-inscribed using a frequency-doubled argon-ion laser emitting at 244 nm and a 1090 nm period uniform phase mask tilted in the plane perpendicular to the incident beam. Irradiated OFs were then annealed at 100 °C in an oven overnight.

2.2.2 Gold coating deposition and thermal annealing

OFs containing TFBG were cleaved beyond the grating location to use them in reflection mode. After cutting the fibers, they were inserted in a vacuum gold sputtering machine to add around 50 nm of gold on each side of the TFBG. They were placed afterwards during 2 hours into a 200 °C thermal resistance to anneal the gold layer. This step ensures a good adhesion of gold on the OF surface. OFs were then washed using absolute ethanol during 30 minutes and dried under N_2.

2.2.3 Optical fiber functionalization

Gold-coated TFBGs were immersed in S_2-PEG_6-COOH (2 mM in ETOH) during 16 hours at room temperature in the aim to create a self-assembled monolayers (SAM). The OFs modified by the self-assembled monolayers of thiols have then undergone an absolute ethanol washing step during 30 minutes and dried under N_2. OFs were then brought into NHS 0.1 M/ EDC 0.5 M diluted into miliQ water during 2 hours at room temperature. Following this, OFs were rinsed with PBS then immediately immersed in AbCK17, at 20 µg/mL (PBS, pH 7.4) during 1h30 at room temperature. They were then rinsed with PBS buffer.
Finally, a blocking step using 5% BSA (w/v) in PBS was applied during 1h30 at RT. Gold-coated OFs were rinsed again with PBS buffer and stored in dry conditions at 4 °C before use.

2.3 Surface Plasmon Resonance measurements

A reflection mirror was deposited on the cleaved extremity of AbCK17 functionalized TFBGs using dedicated silver glue before measurements. OFs were then placed into the POM-packaging and connected to a light polarizer. OFs was then connected to a MicronOptics interrogator, as illustrated in Fig. 1.A. The same set-up was used for every samples tested in this study. Thus, the packaged OF immunosensor was inserted on samples including, PBS buffer, polyacrylamide gels as well as human biopsies. The SPR-TFBG full spectra were recorded for each experiment. However, the analysis of the data only focuses on the most sensitive mode among the spectral comb, according to our previous studies (Caucheteur et al., 2013). To compensate any baseline fluctuations during the experiments, a peak-to-peak analysis of each mode was performed. The resulting values correspond to the amplitude shifts reported in the following.

2.4 Fabrication of the packaging

The packaging (Fig. 1.B) consists in a hollow cylindrical needle (Ø in = 1.2mm; Ø out = 1.6 mm) manufactured in Polyoxymethylene (POM) C2521 Hostaform. The body of the packaging was extruded and the needle tip was thermo-molded. In order to expose the biosensor to the surrounding environment, a window was laser-cut beyond the packaging tip. A stainless steel crimping collar was laser-machined to maintain the biosensor in a fixed position. The external diameter of the packaging is of 1.6 mm with an inner diameter of 1.2 mm. The manufacturing of the packaging was sub-contracted to the company TRANSLUMINAL in Pompey, France.

2.5 Surface characterization

2.5.1 Atomic Force Microscopy

A surface analysis was carried out by Atomic Force Microscopy (AFM) according to the protocol mentioned in our previous work (Ribaut et al., 2016). An ICON (Brucker) was used to scan in QNM mode in air, directly on the top of the optical fiber surface. This time, AFM images were recorded at each step of the bioreceptor functionalization (Au, SAM, AbCK17) with a scan area of 500 nm^2. Images were analyzed by Nanoscope software according to the same treatments.

2.6 Polyacrylamide gel production

Polyacrylamide gels were prepared by co-polymerization of acrylamide as the monomer with the bis-acrylamide as the crosslinker in aqueous media. Gels at desired percentage (Gel% between 4% and 20%) were prepared from the commercial 30% (w/v) acrylamide/bis-acrylamide mixture, by dilution in PBS buffer. The amount of crosslinker was then fixed for every sample at 3.3% (w/v). Polymerization is initiated by the redox system made of APS/TEMED. Briefly, the diluted acrylamide/bis-acrylamide solution was placed at room temperature into a vacuum flask to remove oxygen under stirring. Then, a freshly prepared APS solution at 10% (w/v) in PBS was added to reach the final concentration of 0.05% (w/v). The polymerization only started by adding TEMED (0.05 % w/v in final solution). After 2 minutes under stirring, the solution was placed into Eppendorf tubes (0,5 mL/sample). Polymerization was completed after 15 minutes at room temperature. Gels were then stored at 4 °C by adding PBS buffer on the top to avoid drying.

The gels encapsulating proteins (CK17, FBS, BSA) were prepared according to the same protocol. The proteins were just added homogeneously at the beginning of the protocol, into the acrylamide/bis-acrylamide mixture, prior to the polymerization reaction. These gels were then stored at 4 °C with PBS buffer. Proteins CK17 diffuse through the polyacrylamide gel due to the porosity of these hydrogels as explained in the work of Patras (Patras et al., 2000).

<Insert Fig. 1>

3. Results and discussion

3.1 Immobilization of CK17 antibodies on the OF surface

The sensitivity of the packaged OF immunosensors is directly linked to the amount of immobilized antibodies AbCK17 on their gold-coated surface. These polyclonal antibodies have been selected as bioreceptors due to their high affinity with CK17. Experiments have thus been carried out in order to optimize AbCK17 immobilization on the optical fiber. The molecular edifice that composes the bioreceptor includes a first self-assembled monolayer of thiols (SAM) (Ribaut et al., 2016). Even though the chemistry based on the peptide bond between SAM and antibodies is the same, it has been tested again with the new antibodies AbCK17 used for this study. In fact, manipulation of biomolecules is very delicate and it is well known that it depends on various parameters such as buffer pH, temperature or incubation time. As a consequence, atomic force microscopy (AFM) measurements have been performed at each step of the molecular edifice elaboration. Analysis of the 3D images, illustrated in Fig. 2, clearly depicts the presence of big clusters only after the antibodies (20 µg/mL) incubation stage. Further study of the Fig. 2.B put in evidence piles of the size of 15 nm which match with the size of antibodies. The roughness value (Ra) confirms the modification of the surface with large molecules by increasing from 1.25 nm to 3.03 nm, before and after the bioreceptor fabrication respectively. This experiment proves the stability and the effectiveness of the self-assembled monolayer made of thiols that is used in the aim to immobilize antibodies covalently on the gold surface.

<Insert Fig. 2>

3.2 Detection of CK17 biomarker in buffer

Gold-coated OFs were used as a dip probe in order to be inserted in the packaging (Fig. 1.A), which explains that reflected signals were recorded during these experiments. First measurements performed in simple PBS buffer have ensured that the SPR-TFBG signal was still operational with this new configuration, as illustrated in Fig. 3.A. In fact, the typical reflected spectra obtained with a TFBG immersed in aqueous solution present comb-like cladding modes resonances featuring an SPR signature as indicated in the Fig. 3.A. The polarization controller (Fig. 1.A) placed between the optical fiber and the spectrometer allowed to optimize the signal in order to easily switch between azimuthally-polarized and radially-polarized modes, which was an essential step for the calibration of the signal. The 4 x 1 mm lateral window made in front of the sensitive area (Fig. 1.B) enabled the OF to get access to the sample.

The validation of the packaged OF immunosensor was firstly tested in the presence of increasing CK17 concentrations diluted in PBS buffer (from 10^{-12} to 10^{-6} g/mL). The reflected spectra were recorded after 5 minutes of acquisition time, followed by the monitoring of the most sensitive mode in amplitude. Fig. 3.B displays shifts with increasing CK17 concentrations. The peak-to-peak analysis of this mode has revealed a maximum of 1.2 dB

variation. As a reminder, the evolution of the amplitude, as well as the wavelength, occurs with refractive index changes. Measurements of refractive index (RI) with a refractometer have demonstrated that the addition of CK17 in PBS buffer did not affect the RI of the surrounding medium ($RI_{PBS\ +/-\ CK17}$ = 1.3336 RIU). So, an increase of the amplitude recorded in the presence of CK17 is attributed to the gold-coated surface changes, due to the protein attachment on their corresponding antibodies. This experiment has thus guaranteed the correct operation of the packaged OF immunosensors in liquid samples.

As a consequence, the use of the packaging does not interfere with the selectivity or the sensitivity of the biosensor since CK17 was detected at the very low concentration of 10^{-12} g/mL. If we go further, we notice that the biosensor reaches an identical limit of detection (LOD = 1 pM) as the one achieved in our previous works (Voisin et al., 2015; Ribaut et al., 2016).

<Insert Fig. 3>

3.3 Detection of CK17 biomarker in gel matrix

3.3.1 Optimization of the SPR-TFBG signal

To start, the packaged OF immunosensor was tested on a dilution range (4 to 20%) of polyacrylamide gel concentration in PBS. These gels have the mechanical properties of solids while they are mainly composed of liquids providing them a high degree of elasticity. This characteristic is mainly due to its crosslinked configuration. It is also important to note that the penetration into gels, even for the lowest dilutions considered in this work, required inevitably the use of the packaging due to the stiffness of the hydrogels, as displayed in Fig. 1.C. The sharp end of the packaging was therefore of particular importance during insertions (Fig. 1.B). The recorded spectra, illustrated in Fig. 4.A, highlight the presence of the SPR attenuation (radial polarization or P mode) in gel samples until 16% dilution. These experiments have clearly demonstrated, for the first time, the feasibility to use gold-coated TFBGs in soft matters. The presence of the SPR attenuation in diluted gel matrix could be explained by the nature of the samples. Polyacrylamide gels, produced by the polymerization of acrylamide and cross-linked by methylene bis-acrylamide, form a chemical inert gel network including numerous pores that are able to trap solvent and large molecules. The porosity and thus the density of the gel matrix depend of the polymerization conditions and monomer concentrations. Furthermore, Fig. 4.A shows the disappearance of the SPR signal for the 20% sample, which confirms that excitation of the SPR signal cannot occur in too dense medium. In light of these results, the reference of 10% concentrated polyacrylamide gel was set to mimic tissues.

In the following, the stability of the biosensor response has been tested. In fact, as explained previously, the insertion of the packaged OF immunosensor inside the sample

provokes a slight folding of the packaging. Therefore, functionalized gold-coated OFs have been consecutively inserted in the reference gels. Fig. 4.B displays the superposition of the most sensitive modes recorded at each insertion. The shift observed after 7 successive measurements was calculated to be 0.16 dB, which may be regarded as insignificant. The stability of the SPR-TFBG signal despite the insertion force during multiple penetrations confirms the robustness of the packaging and its crucial role in the OF protection.

<Insert Fig. 4>

3.3.2 Selectivity of the packaged OF immunosensor towards CK17 in gel matrix

Next experiments have consisted in the encapsulation of cytokeratin 17 protein (CK17) into the porous acrylamide gel network in the aim to test the OF immunosensor selectivity. Let us remark that, compared to measurements in liquid, the mechanical frictions and the uncontrolled diffusion of proteins in the gel matrix could largely modify the biosensor response, such as the background signal or the acquisition time.

The following experiments were performed in gels containing 10% (v/v) of fetal bovin serum (FBS). The addition of fetal bovine serum allowed drawing closer to the physiological conditions while testing the specificity of the bioreceptor. In fact, FBS is a mixture made of plenty of proteins which can randomly interact with the gold surface. To avoid such non-specific interactions, a blocking step by immersion in a 5% (w/v) BSA solution was carefully performed on each immunosensor. Fig. 5.B reveals a biosensor response up to 0.56 +/- 0.23 dB in the presence of FBS. An evolution of the amplitude can be explained by a modification of the OF surface or by the change of the surrounding medium RI, what might be the case here since RI increases in the presence of FBS (ΔRI = 0.0008 RIU).

Measurements were then carried out in the presence of CK17 at 5 x 10^{-7} g/mL. Unlike the FBS, addition of CK17 even at high concentration into gels doesn't modify their RI, according to the refractometer data. An evolution of the SPR-TFBG spectrum would thus only correspond to changes of the OF surface. Fig. 5.A, which presents the most sensitive mode of the biosensor response measured with and without CK17 encapsulated in the gel, clearly depicts a large shift in the presence of CK17 compared to the reference gel signal. The experiment was repeated in triplicates, and the analysis of the most sensitive mode, illustrated in Fig. 5.B., has highlighted an amplitude shift of 4.7 +/- 1.7 dB in the presence of CK17, thereby confirming the AbCK17/CK17 strong link that takes place on the gold-coated surface. This big shift can be explained, firstly, by the high concentration of protein used for this experiment (5 x 10^{-7} g/mL), and secondly by the nature of the reflected signal which is doubled in comparison with the transmitted response. This experiment has thus confirmed the very good selectivity towards the target protein. More importantly, these results

demonstrate the feasibility to detect proteins encapsulated in hydrogel. They also pinpoint that the biosensor does not suffer from the diffusion of biomolecules in 10% porous gel matrix since detections have occurred within a minute after the OF implementation.

Statistics were conducted both on FBS and CK17 experiments. Average data displayed in Fig. 5.B were subjected to the Student test by applying the Shapiro-Test. Calculated p-value was below 0.05, which means that the results are significant, confirming thus the selectivity of the bioreceptor.

3.3.3 Sensitivity of the packaged OF immunosensor towards CK17 in gel matrix

The next step focused on the sensitivity of the immunosensor towards CK17. For this purpose, experiments have been carried out in the aim of testing the biosensor response in the presence of lower concentrations of CK17. In order to fulfill the analysis, the immunosensor was firstly tested in a gel containing a nonspecific protein used as negative control. To do so, BSA was chosen due to its molecular weight similarity with CK17 (66 kDa vs 73 kDa), an important parameter since SPR is mass dependent. Moreover, BSA should have the same diffusion characteristics across the crosslinked polymer.

Fig. 5.C displays the amplitude monitoring of the most sensitive mode for each tested sample. First, the reference signal was recorded in a gel without proteins. Then, the packaged OF immunosensor was inserted in a gel polymerized with BSA at high concentration (1×10^{-6} g/mL), causing a shift of 0.147 dB. It is pertinent to note that the addition of BSA in the sample did not modify the RI of the surrounding medium, as in the case of CK17. Despite the highly concentrated sample of BSA, the immunosensor has shown an insignificant response, characterized by a low shift, and has thus confirmed the specificity of the bioreceptor towards CK17.

Following experiments consisted in the insertion of the same functionalized optical fiber in the porous acrylamide gels polymerized in the presence of CK17 starting from 1×10^{-10} g/mL. The peak-to-peak analysis of the sensitive modes of the SPR-TFBG spectra, presented in Fig. 4.C, clearly depicts a large amplitude evolution, as soon as the bioreceptor was in contact with its corresponding antigen. Fig. 4.C. displays a total shift equal to 1.5 dB at 1×10^{-10} g/mL, which proves the high sensitivity of the bioreceptor.

Yet, a smaller gap of 0.8 dB was then observed for the next sample at 1×10^{-6} g/mL. This result can be explained by the decrease of the immobilized antibodies available on the OF surface during experiments. Consequently, despite the presence of the target CK17 in the surrounding medium, the antibody/antigen bindings reduce, which is reflected by a decrease of the amplitude shift. We can note that for repeated experiments the evolution of the signal has decreased until it reached a threshold which could be explained by the saturation of the immobilized antibodies since they are no longer available for CK17.

The more important information relies on the limit of detection achieved by the biosensor. In fact, once again the biosensor was sensitive for the very first concentration tested at 10^{-10} g/mL. It is also interesting to note that encapsulation of the target proteins into the crosslinked polymer did not alter the rapidity of the biosensor response since the acquisition time is identical in liquid or in gel matrix.

<Insert Fig. 5>

3.4 Detection of CK17 biomarker in human tissue

Preliminary tests on human lung biopsy for the *ex-vivo* detection of CK17 were finally performed, as illustrated in Fig. 6.A. The packaged OF immunosensor, which was functionalized with AbCK17 as previously described, was firstly tested on the healthy lung tissue. Here, it is worth noting that this *ex-vivo* experiment allowed the confirmation of the easy manipulation and insertion of the biosensor even in tissue. The corresponding recorded radially-polarized mode spectrum, displayed in Fig. 6.B, clearly put in evidence the attenuation of few modes (λ≈1542 nm), which confirms the correct SPR excitation. The monitoring of the most sensitive mode, displayed in Fig. 6.C, has then revealed an unreliable evolution of the amplitude with a variation of +/- 0.11 dB. This behavior mainly indicates that no detection was monitored. Moreover, this insignificant variation proved that no non-specific adsorption occurred around the OF despite the complexity of the sample which proves again the efficiency of the BSA blocking step performed at the end of the surface functionalization.

The same OF immunosensor was then incorporated in the tumoral tissue of the lung biopsy. Despite the stiffness of the tumoral sample in comparison with the healthy one, the packaging allowed inserting the biosensor while maintaining the SPR-TFBG signal. The biosensor response behaves differently compared to the healthy tissue. In fact, Fig. 6.D depicts a linear increase (R^2=0.98) of the amplitude reaching an amplitude shift of 0.61 dB within 10 minutes which is attributed to the CK17 detection. This preliminary test performed in a fresh human biopsy has then revealed a positive *in situ* and on-line biosensor response for the specific cytokeratin 17 detection.

<Insert Fig. 6>

4. Conclusion

For the first time, we have demonstrated that the accurate detection of biomarkers in soft matters including tissues can be performed with plasmonic optical fiber grating

immunosensors. A dedicated packaging made of a biocompatible polymer has been manufactured with the aim of embedding the sensors that were then successfully used as dip probes for the detection of cytokeratin 17 protein. This study has first proven that the immunosensor was operational in liquid environment reaching a good limit of detection. The optical response of the SPR-TFBG tested in porous gel matrix has put in evidence, for the first time, the presence of a stable SPR signal despite the non-liquid nature of the sample. A set of experiments have thus been performed in the presence of CK17 trapped in the crosslinked polymer network. The monitoring of the reflected spectra has confirmed the specificity of the response as well as its good selectivity towards the target proteins. The packaged optical fiber immunosensors combine the high sensitivity of the SPR-TFBG technology with the small size of the optical fiber and henceforth the robustness thanks to the use of a biocompatible packaging. Therefore, the developed biosensors have successfully allowed non-invasive *ex-vivo* measurements on human tissue providing from lobectomy with a positive biosensor response in tumoral sample. In consequence, this biosensor platform opens a new way for the *in-situ* and on-line detection of biomarkers in tissues.

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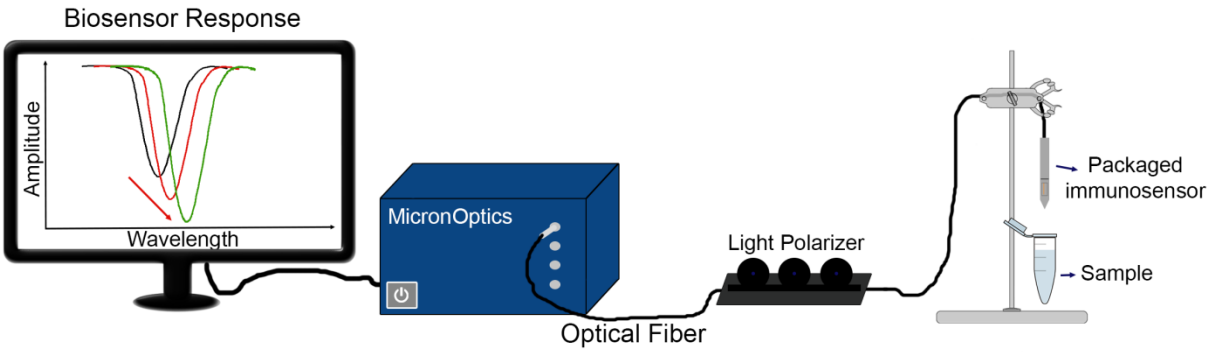
Figures

A



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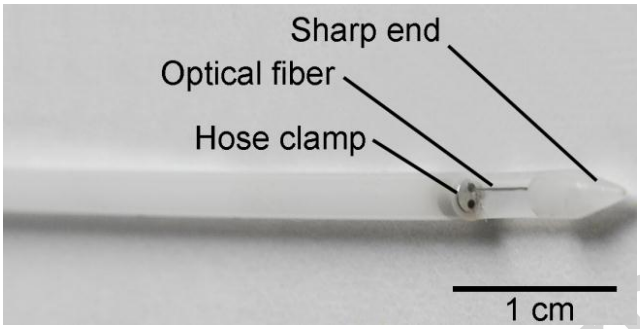

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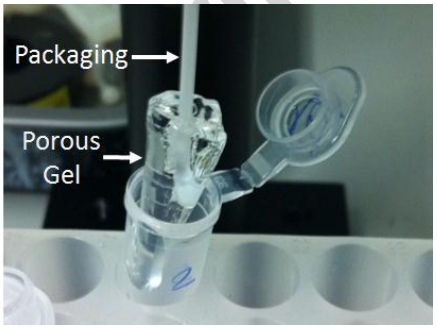
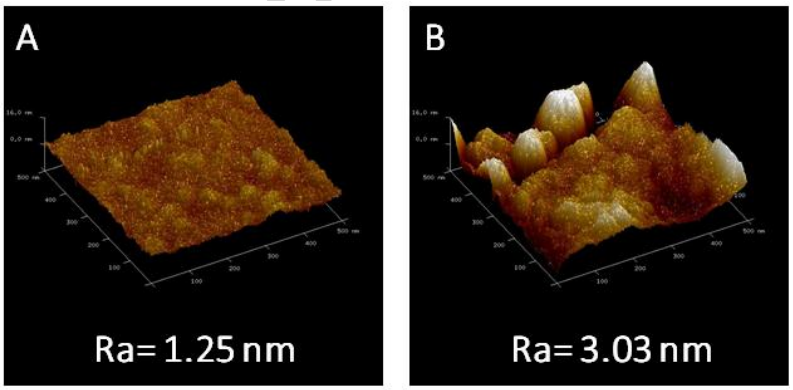


Fig. 1.



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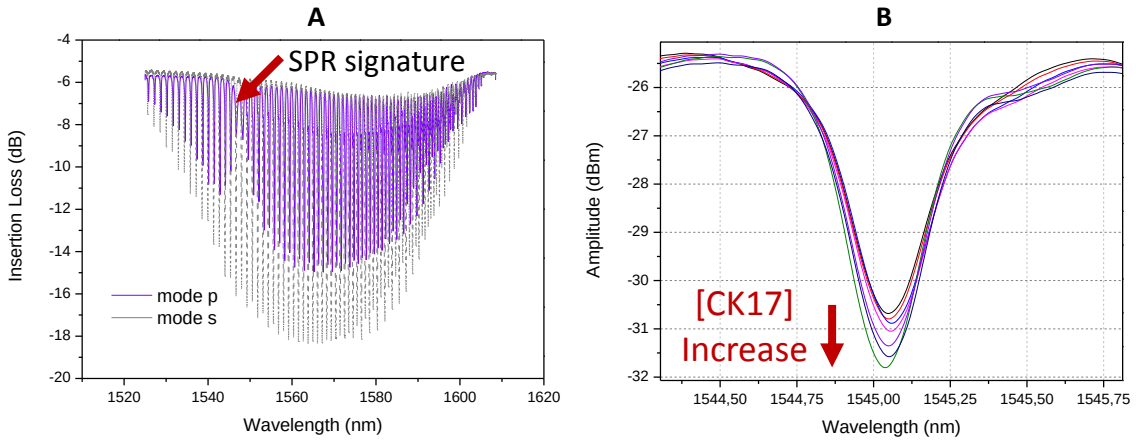

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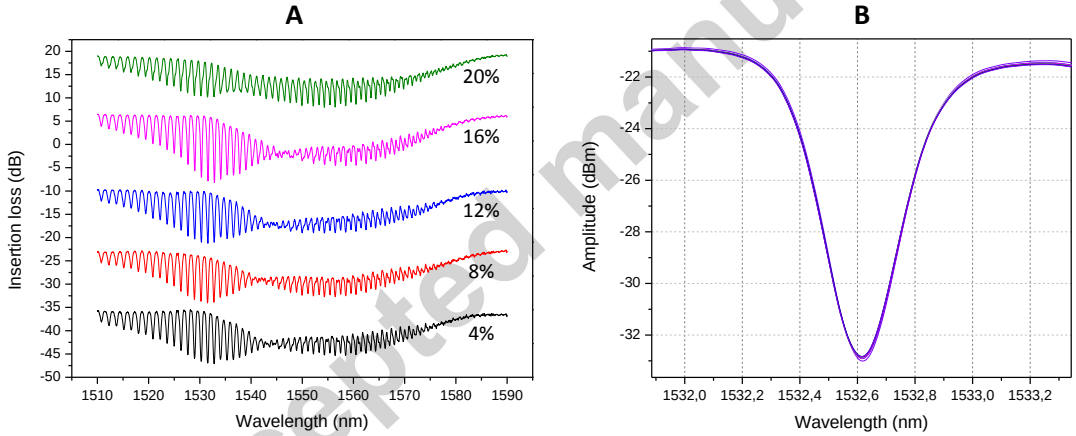

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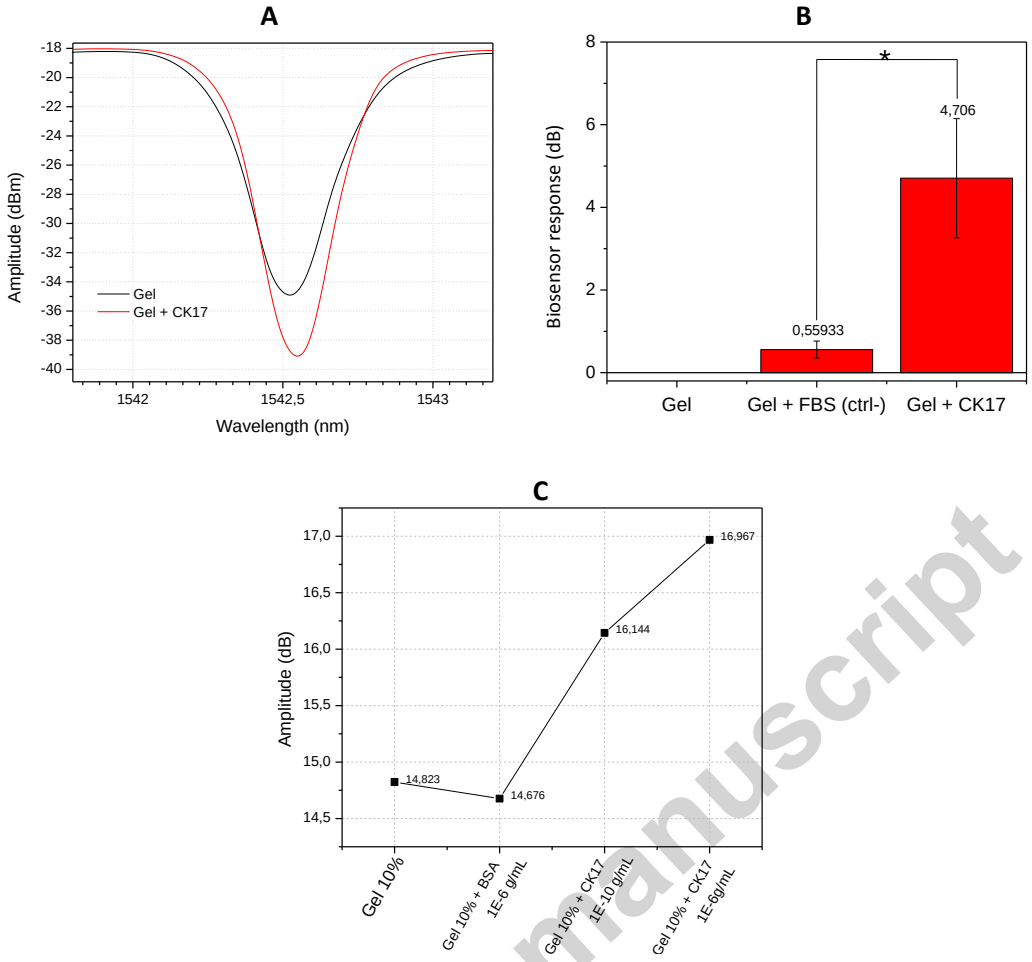

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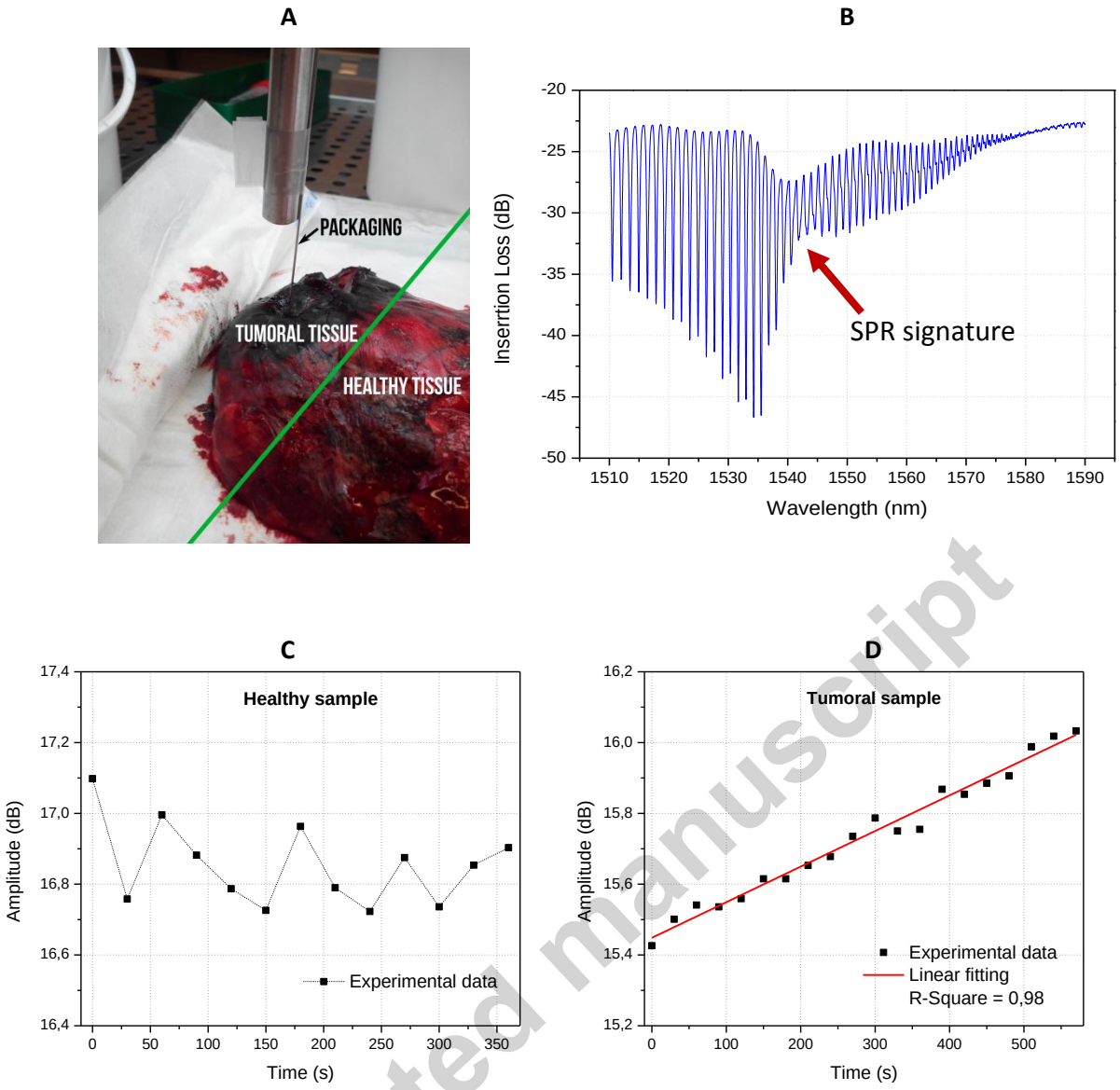

Fig. 6.

Captions

Fig. 1. (A) Schematic overview of the set up used for the SPR-TFBG measurement, and (B) a cliché of the packaged optical fiber immunosensor including the biocompatible packaging with an OF inserted in the hose clamp. (C) Picture of the packaging inserted in a polyacrylamide gel sample.

Fig. 2. AFM 3D images obtained in QNM mode in air on (A) 50 nm thickness gold-coated on optical fiber and (B) after the functionalization of the gold coated surface with SAM/AbCK17.

Fig. 3. (A) Reflected spectra obtained with the packaged OF immunosensor by polarizing the light in PBS buffer. (B) Monitoring of one sensitive mode increasing CK17 concentrations (from 10^{-12} to 10^{-6} g/mL) diluted in PBS buffer.

Fig. 4. (A) SPR-TFBG spectra recorded in polyacrylamide gels at different concentrations. (B) Shift of the most sensitive mode during 7 implementations of the biosensor onto polyacrylamide gel in absence of protein target.

Fig. 5. (A) Shift of the most sensitive mode of the SPR-TFBG signal recorded in polyacrylamide gels +/- CK17. (B) Biosensor response recorded in polyacrylamide gel complemented with FBS and in presence of CK17. (C) Amplitude monitoring of the most sensitive mode recorded while increasing CK17 concentrations crosslinked in polyacrylamide gel.

Fig. 6. (A) Picture of the immunosensor incorporated in the tumoral tissue of the human lung biopsy, and (B) SPR-TFBG spectrum recorded when the immunosensor is inserted in the tissue (C) Amplitude monitoring of the most sensitive mode obtained on the healthy tissue of the biopsy and (D) on the tumoral tissue of the biopsy.

Highlights:

- Immunosensing experiments with cytokeratins 17 (cancer biomarkers for lung cancer diagnosis) confirming the high sensitivity of plasmonic near-infrared optical fiber gratings biosensors and demonstrating their reversibility and robustness.
- Experiments conducted in porous gel matrices with biosensors embedded in a specially designed packaging
- CK17 detection in a fresh biopsied lung tissues using the aforementioned biosensing platform