Biomarker detection using packaged plasmonic optical fiber

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The biomarker detection is an essential step for early cancer diagnosis. In that way, this work presents the development of an innovative plasmonic optical fiber immunosensor. A gold-coated optical fiber with a photoinscribed Tilted Fiber Bragg Grating (TFBG), embedded in a specifically designed packaging provides enough stiffness to penetrate into soft matters and human tissues. This biosensor is able to detect biomarkers thanks to the excitation of a surface plasmon wave. For the first time, we demonstrate the detection of a proteic target at very low concentration in hydrogels, what is an important milestone towards medical diagnosis.

Introduction

Early diagnosis of cancer is essential to ensure a high remission rate. Nowadays, for some kinds of cancers, tumorous masses are already present while the diagnosis is only achievable. These affected tissues are also needed to analyze and evaluate the evolutionary stage of the pathology [1]. Unfortunately, cancers are not easily visible precociously, what explains some delayed findings with severe or fatal consequences. Furthermore, to avoid misdiagnosis, numerous biopsies are sometimes carried out in order to confirm or infirm the clinical verdict, which results in a large amount of unnecessary surgery causing pain and discomfort to patients.

In that way, we propose to detect the presence of biomarkers at early stage and to distinguish different types of cancers using a specific set of biomarkers. Actually, when cells are disordered and become cancerous, they can produce a lot of analytes that are not present in healthy cells. These production differences between healthy and tumor cells can be spotted to determine the pathology [2]. However, the amount of targeted analytes is very weak at the beginning of a cancer formation. So, it is necessary to develop a new technique able to detect the presence of these analytes with high affinity and specificity.

A lot of reliable and well-known techniques are used to detect biomarkers since decades (ELISA, PCR, IHC, SPR, etc.) [3,4] but none of them are able to do online and *in vivo* molecular measurements. Anyway, many research programs investigate new methods to diagnose cancers or diseases at early stages. As a contribution to these existing techniques, we decided to develop a challenging project using the Surface Plasmon Resonance (SPR) phenomenon on optical fiber (OF). The surface excitation of the plasmon is allowed through a photo-inscribed Tilted Fiber Bragg Grating (TFBG) [5]

and the specific biomarker recognition is possible thanks to the specific antibodies immobilization on the OF surface. If the biomarker-targets are present in a suspected tumoral tissue, they can interact with the immobilized antibodies and provoke a signal variation that is monitored during the infrared spectrum analysis [6].

Finally, to implement this project in real conditions, we consider that the final step of the development of this biosensor would be to perform *in vivo* measurements for lung cancer, considered as an adequate model to test our catheteric concept.

Material and Methods

Single-mode optical fibers (Corning SMF-28) were used to prepare each biosensor. TFBGs were inscribed with an external tilt angle of 7° in the OF-core previously hydrogenated. 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.

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 50 nm of gold on each side of the TFBG. After that, they were placed during 2 hours into a 200 °C thermal resistance. OFs were then washed using absolute ethanol during 30 minutes and dried under N_2. Gold-coated TFBGs were afterwards immersed in S_2-PEG_6-COOH alkanethiols (2 mM in ETOH) during 16 hours at room temperature. They were then washed with ethanol during 30 minutes and dried under N_2. OFs were also brought into a mixture of N-hydroxysuccinimide (0.1 M/ 1-Ethyl-3-(3 Dimethylaminopropyl Carbodiimide Hydrochloride) 0.5 M diluted into miliQ water during 2 hours at RT. Following this, OFs were rinsed with PBS then immediately immersed in anti-CK17 antibodies at 20 µg/mL in Phosphate Buffer Saline (PBS, pH 7.4) during 1h30 at room temperature (RT). They were then rinsed with PBS. Finally, a blocking step using 5% (w/v) Bovin Serum Albumin (BSA) in PBS was applied during 1h30 at RT. Gold-coated OFs were finally rinsed with PBS and stored in dry conditions at 4 °C before use (Figure 1). Before each experiments, the biosensors were inserted into a biocompatible polymer packaging to mimic experimental conditions with a catheter - (Ø in = 1.2 mm; Ø out = 1.6 mm) manufactured in Polyoxymethylene C2521 Hostaform.

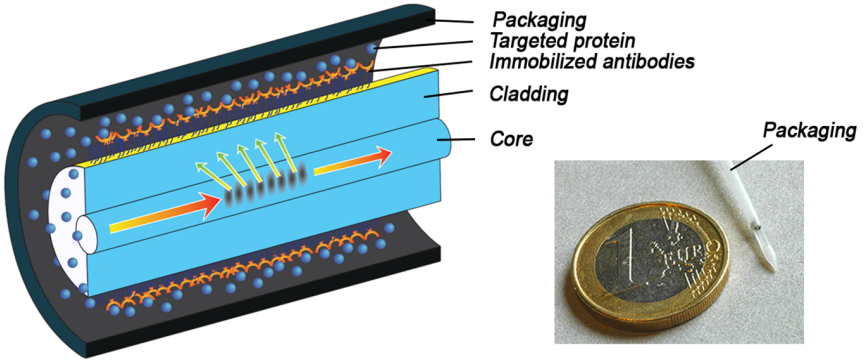

Figure 1. Scheme of the SMF-28 TFBG-OF functionalized with antibodies directed against its corresponding proteins. The packaging (Ø 1.6 mm) has an interaction window and a hard tip to penetrate tissues, as presented on the right picture.

Polyacrylamide gels at different concentrations (Gel Dilution: 4% to 20%) were also prepared from a 30% acrylamide/bisacrylamide (29:1) by dilution in PBS buffer. These non-liquid samples were used to mimic mechanical stresses existing in human tissues. Different concentrations of biomarkers were also added homogeneously into these gels before their polymerization at ambient temperature.

Experiments and Results

The first experiment consisted in demonstrating the presence of a SPR phenomenon using a reflection-type TFBG-OF inserted into a packaging. The figure 2A displays the two polarization modes with the presence of the SPR signature for the p-mode. This test confirmed the presence of a SPR signature in PBS-buffer. Then, the increasing CK17 concentrations in this PBS buffer (from 10^{-12} to 10^{-6} g/mL) showed a variation of the amplitude of the most sensitive mode of the spectra (Figure 2B).

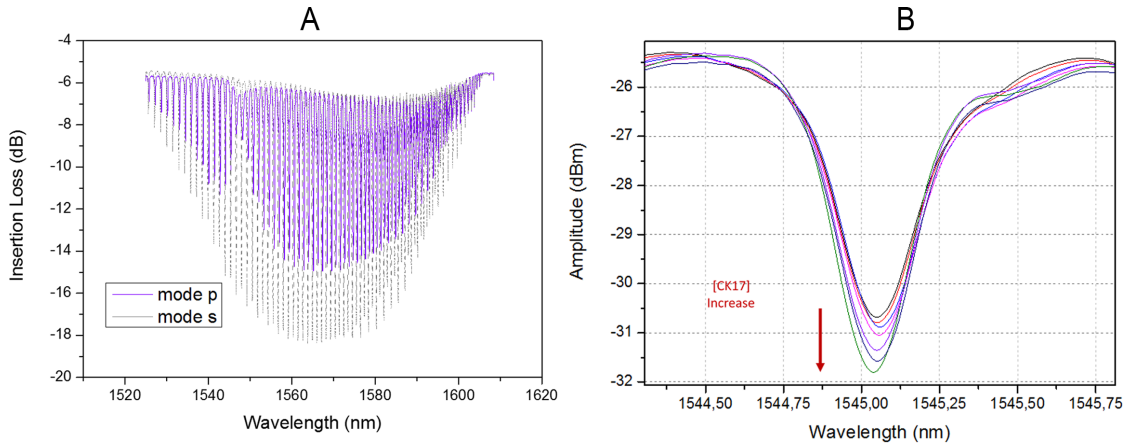

Figure 2. A. Reflection spectrum obtained with TFBG-OF in PBS. B. Sensitive mode variation with increasing CK17 concentrations (from 10^{-12} to 10^{-6} g/mL in PBS).

After that, the packaged immunosensors were tested in a range of (4 to 20%) polyacrylamide gels in PBS. The penetration into these gels requires the presence of the packaging due to their stiffness. A good SPR signature was recorded up to 18% polyacrylamide gels. At higher polyacrylamide concentrations, no SPR phenomenon was observed. So, the use of 10% polyacrylamide gels was chosen to allow the SPR phenomenon on one hand, but also to approach the mechanical properties existing in human tissues on the other hand.
Moreover, specificity tests were performed using solutions and gels including 10% of fetal bovine serum (FBS) containing different kind of proteins and mimicking physiological conditions. With the presence of FBS in solution or in polyacrylamide gels, no signal variation was observed confirming the only recognition of CK17 proteins, which are not present in FBS.

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 does not modify their refractive index, according to the refractometer data. An evolution of the SPR-TFBG spectrum would thus only correspond to changes of the OF surface. The most sensitive modes of the biosensor response, measured with and without CK17 encapsulated in the gel matrix, clearly depict a large shift in the presence of the targeted biomarker

compared to the reference signal (Figure 3). The experiment was repeated in triplicates, and the analysis of the most sensitive mode has highlighted an amplitude shift of 4.7 +/- 1.7 dB in the presence of CK17. This big shift can be explained by the high concentration of proteins used for this experiment (5 x 10^{-7} g/mL), but also 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. Statistics were conducted both on FBS and CK17 experiments. Average data displayed in Figure 3 were subjected to the Student test (p-value < 0.05).

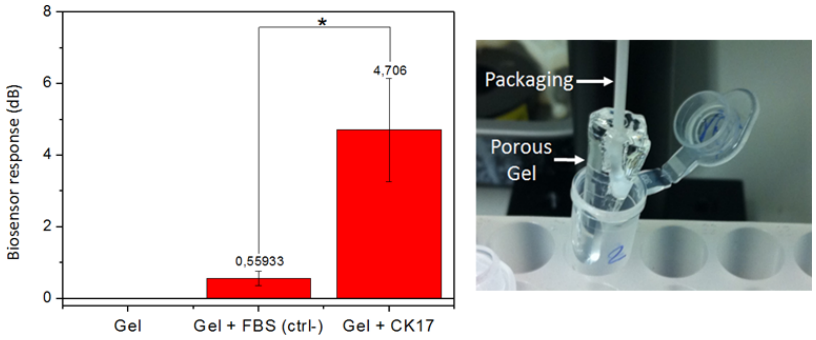

Figure 3. Average amplitude variation of the most sensitive modes of 3 AbCK17 TFBG-OF inserted in: first, a 10% polyacrylamide gel; then a 10% gel containing FBS as negative control ($ctrl^-$); and finally a 10% gel containing 5 x 10^{-7} g/mL of CK17.

Conclusion

The first results obtained in this study show the possibility to develop a new era of *in vivo* biosensing systems coupling the thinness of optical fibers and molecular detection. Indeed, our *in vitro* results demonstrate the use of the SPR phenomenon and the detection of CK17 proteins at low concentration in non-liquid samples. These results are a step forward for the *in vivo* measurements. Moreover, the antibodies immobilization can be adapted according to the biomarkers of interest, making this tool a modular platform for the online and *in situ* diagnosis of diseases or cancers.

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