Cancer cells detection using multiresonant optical fiber biosensors

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The detection of circulating tumor cells (CTCs), which are responsible for metastasis in several forms of cancer, represents an important goal in oncological diagnosis and treatment. CTCs remain extremely challenging to detect due to their low concentration (1-10 cells/mL of blood). In this work, we show an all-fiber plasmonic aptamer-based sensor featuring multiple narrowband resonances in the near-infrared range. Using specific DNA strands anchored on nanometric gold films deposited around tilted fiber Bragg gratings (TFBGs), our biosensors were able to target proteins (namely, human epidermal receptors HER2 and mammaglobin proteins MAM) expressed at the surface of cancerous cells. Our experimental results confirm a label-free and real-time detection of 49 cells/mL within 5 minutes in vitro, while the additional use of aptamers-functionalized gold nanoparticles yielded an amplification of the response. Results were confirmed by microscopic analysis and using non-target cell cultures. Finally, the plasmonic amplification reached a detection of only 10 cancer cells per mL, paving the way for their future development as practical and cost-effective diagnostic tools.

Introduction

Biosensors have known cutting-edge improvements over the last years, especially in terms of miniaturization, versatility, and sensitivity. While well-known and gold-standard laboratory-based techniques such as enzyme-linked immunosorbent assay (ELISA), polymerase chain reaction (PCR) or surface plasmon resonance (SPR) are very popular, trends in point-of-care and low-cost sensors available for a wide public are emerging. This interest depicts the high potential for democratization of new technologies, as a cornerstone for new developments. The recent covid-19 crisis together with diseases such as tropical infections, resurgence of sexually transmitted diseases and abundance of cancers have put in evidence the need for fast and accurate diagnosis tools. The rise in technology readiness level between academic research and industrial needs has also demonstrated that synergy is necessary to improve the efficiency of existing techniques, innovate, and turn a prototype into a market product.

Among these technologies, optical fiber-based biosensors bring singular features such as flexibility, reliability, cost-effectiveness, etc. They also enable the detection of molecules or cells in small volumes and hard-to-reach environments. In this paper, we make use of tilted fiber Bragg gratings (TFBGs) photo-inscribed within standard monomodal telecommunication fibers (SMF-28). The presence of such tilted gratings breaks down the cylindrical symmetry of the fiber and leads to the outcoupling of some narrowband cladding modes at specific wavelengths, depending on the grating parameters (tilt angle, period, length, strength of the grating, etc.). The spectral cladding modes obtained from those structures lead to high precision monitoring by determining the position of mode resonances. Recent advances in this field have also demonstrated that TFBGs can be tracked using top or bottom envelopes, considering minima or maxima from the

envelopes, respectively. Their crossing point is also highly relevant to follow, as recently reported. [1-2]

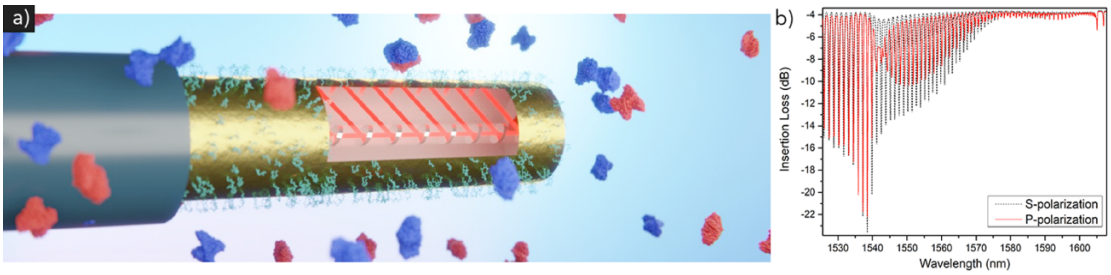

Figure 1. (a) Artistic view of a gold-coated tilted fiber Bragg grating sensor for protein detection (not-in-scale), (b) Spectra obtained with a gold-coated TFBG (S- and P-polarizations).

Materials and methods

Standard telecommunication-grade optical fibers (SMF-28, Cording) were hydrogen-loaded under ~200 bars and 60°C for 30 hours to enhance their photosensitivity. Pieces of this fiber were used to achieve the inscription of permanent tilted fiber Bragg gratings (8° tilt angle) using the phase-mask technique (Noria, embedding an excimer laser at 193 nm, form Northlab Photonics). The phase mask has a period of 1100 nm. After a dehydrogenation at 100°C for one day, a uniform gold film of ~50 nm was sputtered all around the TFBG location (Leica EM SCD 500), yielding the generation of surface plasmon resonance (SPR) in the NIR region. The fiber was connected to a LUNA (optical vector analyzer) with a polarizer to select the P-mode and track the plasmonic evolution. The Bragg wavelengths were aligned on all data to get rid of any parasite fluctuations due to temperature and/or mechanical constraints.

Gold-coated TFBGs were then biofunctionalized using specific DNA bioreceptors (aptamers) targeting mammaglobin proteins (namely, MAMA2) expressed at the surface of breast cancer cells (MDA-MB-415 cell lines), or HER2 proteins. The aptamers were synthesized using phosphoramidite chemistry (MerMade 6 automated DNA synthesizer from BioAutomation, USA) and further thiolated at 3' end. MDA-MB-415, MCF7 and HEK293 cell lines were cultured as target cells and control cells.

Experimental results

Over the last six years, our group has developed biosensors against proteic biomarkers and cells, aiming at their detection at ultra-low concentration and in complex media. Our optical fiber probes were extensively tested for lung cancer biomarkers and were for the first time embedded inside catheters to reach the upper-lobe region of lungs. They were assayed inside pig lungs and resected human tissue to attest the presence of cytokeratin-17 proteins. [3][4] Our devices were also recently tested against circulating breast tumor cells to specifically detect mammaglobin proteins expressed at their surface and freely circulating HER2 proteins.

In the framework of cellular detections, gold-coated TFBGs were functionalized with DNA-bioreceptors called aptamers, specifically designed to target mammaglobin proteins and with a thiol-end to strongly bind on gold and self-organize on the sensor surface (**Figure 2**).

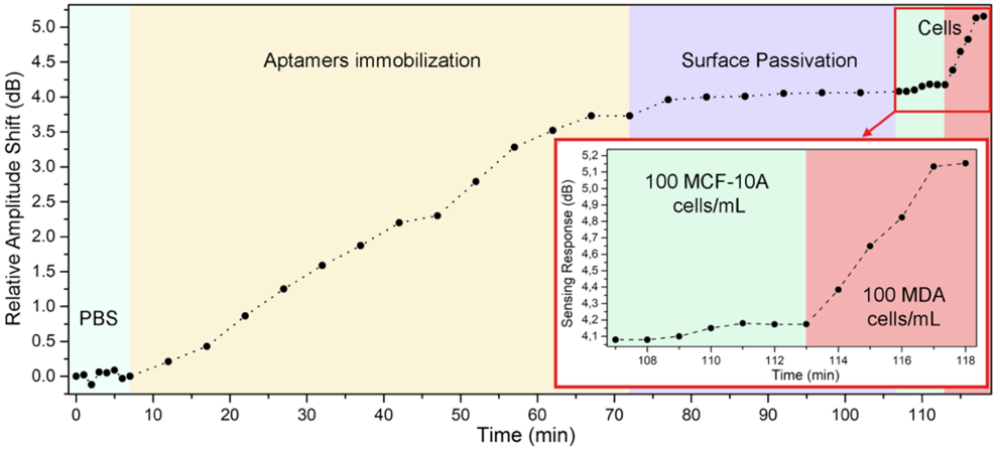

Figure 2. Sensorgram showing the functionalization process (mammaglobin aptamers), blocking (mercaptohexanol), and detection of cells (control cells: MCF-10A and target cells: MDA) using a gold-coated TFBG.

As an example, *in vitro* assays confirm that label-free and real-time detection of cancer cells occurs within 5 minutes. Differential measurements on selected optical resonances were used to process the sensor response and the results were confirmed by microscopy (SEM and fluorescent microscopy). Finally, a limit of detection of 49 cancer cells per mL was achieved in a label-free configuration, with relevant specificity against control cells.

To improve the signal-to-noise ratio and consequently to lower the detection threshold obtained with the previously described label-free configuration, functionalized gold nanoparticles of 10 nm diameters were used to bind with free target proteins remaining on the cells already attached on the TFBGs receptors. Some of these GNPs therefore act as amplification labels and play the role of enhancers due to their inherent properties (mass effect, local refractive index change, and localized plasmonic effects). These observations were performed during the addition of cells expressing mammaglobins on their surface and demonstrated the possibility to detect up to 10 cells per mL in PBS buffer, even after several washing steps (**Figure 3a, 3b and 3c**)[5].

Statistical analyses were also performed on the detection data obtained from 3 different SPR-TFBGs successively immersed in the 10 control cells, functionalized GNPs, and 10 target cells, followed by a second bath of functionalized GNPs, as reported in **Figure 3d** [5].

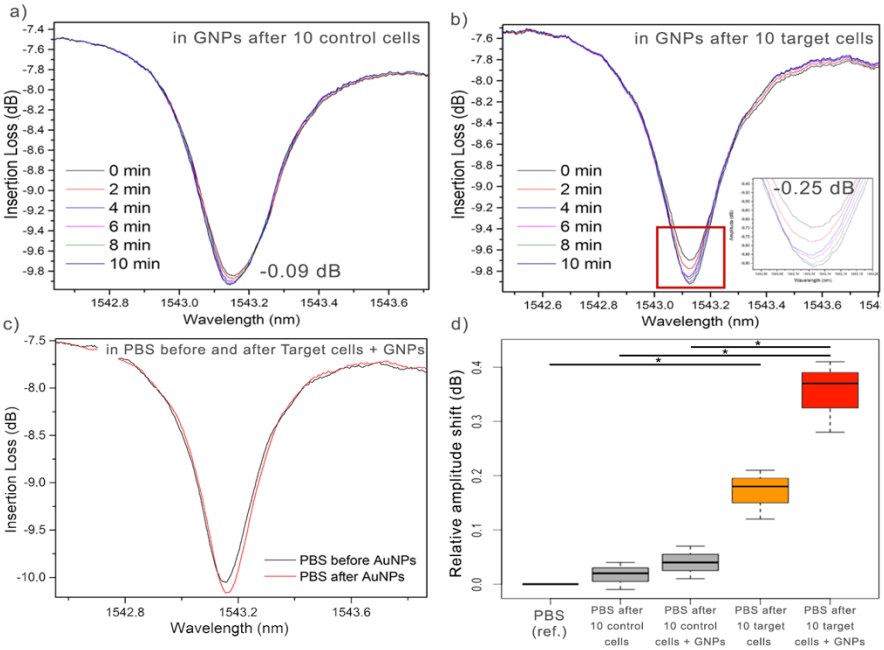

Figure 3. (a) Evolution of the signal in functionalized gold nanoparticles after 10 control cells/mL. (b) Evolution of the signal in functionalized GNPs after 10 target cells/mL. (c) Signal variation in PBS before and after GNPs incubation. (d) Boxplot of the relative amplitude shift in PBS (ref.) and after each tested condition (N = 3, p-value < 0.005, R-studio v1.0.153).

Conclusion

The detection of tumor cells is a hot topic for the development of accurate and highly sensitive diagnostic tools. In this paper, we present the use of specific aptamers directed against target proteins located at the surface of cancer cells. We show that a limit of detection of 49 cells/mL was achieved using a label-free detection and the amplification using GNPs improved the detection down to 10 cells/mL. The specificity of the probes was tested with different cell lines and demonstrates the relevance of optical fiber-based biosensors in this context of circulating cells detection at low concentration.

References

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