CONSIGLIO NAZIONALE DELLE RICERCHE – ISTITUTO DI FISICA APPLICATA “NELLO CARRARA”

Detection of Alzheimer's Tau protein using nanocoated fiber biosensors

“Short–Term Mobility” 2017

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Scientific report on the research activity developed at the Department of Electrical and Electronic Engineering, Institute of Smart Cities (ISC) belonging to the Public University of Navarra (UPNA) (Pamplona, Navarra, Spain) during the period 8 – 28 February 2018

Summary

1. Introduction

Optical fiber sensors have a large impact in the fields of life science research, drug discovery and medical diagnostics.[1] The recent advances in nanotechnology and photonics have led to a new generation of nanotools, capable of probing analytes at incredibly low limit of detection (LOD): it has already been demonstrated that fiber-based biosensors can detect biochemical targets and proteins below pM LODs.[2] The photonic element chosen as substrate for the immobilization of the biorecognition element was a D-shaped single-mode optical fiber, integrated into a thermo-stabilized microfluidic system.

Alzheimer's disease (AD) is the most common neurodegenerative disease and is characterized by a progressive loss of cognitive function, leading to dementia. The two neuropathological hallmarks of AD are extracellular senile plaques composed of aggregates of amyloid-beta protein (Ab) and intracellular neurofibrillary tangles (NFTs) composed of aggregates of the hyperphosphorylated microtubule-associated tau protein (TP).[3] Tau plays a role in promoting the assembly and maintenance of microtubules through its microtubule-binding domain. The capacity of tau to bind microtubules and promote stabilization and assembly is negatively regulated by its phosphorylation, particularly in and around the microtubule-binding domain. Under pathological conditions, such as AD and others tauopathies, tau becomes hyperphosphorylated, resulting in reduced affinity for microtubules and self-aggregation into abnormal filaments, leading to formation of NFTs.[4] Among the different approaches use in the recent literature, such as home-made developed ELISA tests, Western blotting (WB), electrochemical impedance spectroscopy (EIS),[5] surface plasmon resonance (SPR),[6] it is still hard to find a reliable optical platform able to guarantee a LOD down to the clinical cut-off value of TP in cerebro-spinal fluid (CSF), which is 4.3 pM (hundreds of ng/L).

Particular interest for this STM-project has been focused on preventing and challenging the AD by means of the study and monitoring of TP using a fiber-optic technology platform with special nanometric coatings. The main objective of the STM-project is to implement and characterize a receptor-analyte (antibody-antigen) label-free immunoassay able to effectively monitor TP variations, one of the primary biomarker of the onset of AD. In particular, based on previous experiences of the two Institutes involved in, both the thermo-stabilized microfluidic system and the model assay used, which encompasses previous-performed experiments that involved other biomolecules, have been designed, implemented and tested at IFAC; whilst the fiber-optic sensors coated with tin dioxide and based on the excitation of lossy mode resonance (LMR) phenomenon have been designed and fabricated at UPNA.

As preliminary study, before detecting low concentrations of TP, high concentrations of TP were tested in buffer solutions. The receptor concentration was also changed in order to understand the antibody concentration value able to guarantee an optimum surface coverage of the sensing portion of the fiber-optic device.

2. The fabrication of nanofilm SnO_2-coated fiber sensor

The nanofilm of tin dioxide (SnO_2) used to coat the fiber sensors was directly deposited at the Department of Electrical and Electronic Engineering, Institute of Smart Cities (ISC) of Public University of Navarra (UPNA), using a DC sputter machine (ND-SCS200 from Nadetech S.L.). The target material, i.e. SnO_2 with a 99.99% of purity, was purchased from ZhongNuo Advanced Material Technology Co. It was demonstrated that SnO_2 is the best coating to develop high sensitive LMR sensors when compared with other oxides, like indium tin oxide (ITO)[7] or indium gallium zinc oxide (IGZO).[8]

As the optical fibers used to implement the sensor concerns, D-shaped single-mode fibers (SMFs) purchased from Phoenix Photonics were utilized. Those consisted of a standard SMF, like Corning® SMF-28, with a side-polished length of 17 mm (see Figure 1, left). The polished surface of the optical fibers, henceforth called sensitive region, was then coated with the thin film. The parameters set during the deposition process of the SnO_2 target were a partial pressure of argon 9×10^{-2} mbar and a current intensity of 90 mA. All the steps devoted to the fabrication of the fiber sensor were developed at UPNA.[7,8]

A field emission scanning electron microscope (FESEM), model UltraPlus FESEM from Carl Zeiss Inc., with an in-lens detector at 3kV and an aperture diameter of 30 µm, was used to measure the coating thickness. Figure 1 (right) shows a SEM image of the sensor cross-section at the final fabrication step. By repeating several time the deposition process, a film thickness of roughly 160 nm was measured. The reproducibility of the film deposition was estimated to be around 10-15 nm.

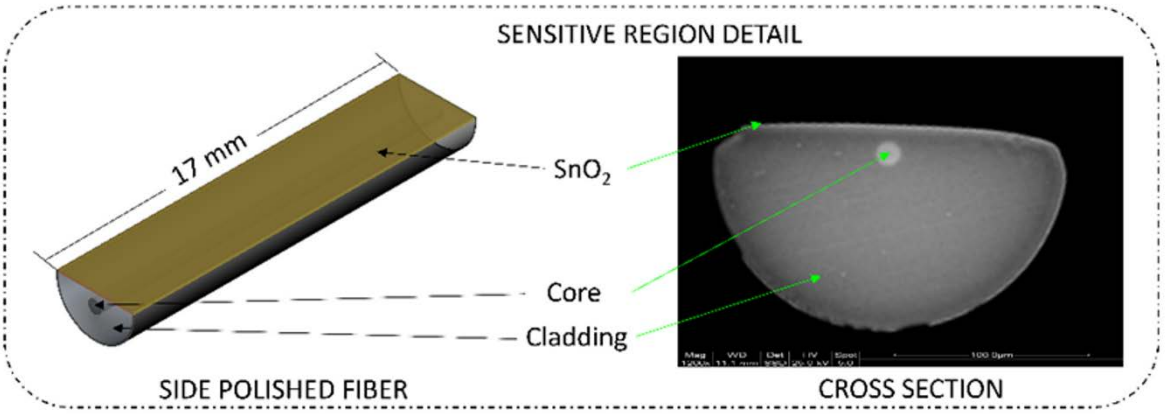

Figure 1. (left) Sketch of the sensitive fiber region consisting of a 17 mm-long D-shaped fiber portion coated with a nm-thick film of tin dioxide, SnO_2. (right) SEM image of the sensor cross-section at the final fabrication step.

3. The model assay used to detect Tau protein in buffer matrix

The interest for this project was focused on the detection of TP, a clinically relevant biomarker for AD. In particular, a model assay protocol was developed to test the detection capability of the fabricated device. First of all, the sensitive region was functionalized in order to have free carboxylic groups for binding the receptors. To do that, a poly(methyl-methacrylate) polymer (Eudragit L100) was purchased from Evonik Degussa GmbH and then used. The sensitive region of the device was immersed in 2 mM (0.04% w/v) Eudragit L100 in ethanol for 1 minute and subsequently was left to dry in the air for about 15 minutes in order to let the solvent completely evaporate.

According to previous publications on the subject,[9-11] the following steps were implemented after the sensor was place inside a thermo-stabilized microfluidic system:[12]

I. activation of free carboxylic (-COOH) groups by means of EDC (40 mg mL^{-1}) and NHS (6 mg mL^{-1}) for 30 minutes (flow rate of 40 µL min^{-1});

II. immediate covalent immobilization of the anti-tau antibodies (Tau46) on the activated surface, by injecting a solution of 500 mg L^{-1} Tau46 in PBS for 1 hour (flow rate of 5 µL min^{-1});

III. washing with PBS buffer to remove the unreacted antibodies for 15 minutes (flow rate of 5 µL min^{-1});

IV. surface passivation with 1% (w/v) BSA in PBS for 30 minutes (flow rate of 5 µL min^{-1}) in order to reduce/eliminate the non-specific adsorption of the antigen on the sensitive region surface.

V. assay completion by injecting solutions of the specific analyte (Tau441) in increasing concentrations that ranged from 0.01 mg L^{-1} up to 50 mg L^{-1} (flow rate of 5 µL min^{-1}).

Figure 2 sketches all the steps to prepare the biorecognition layer and the model assay.

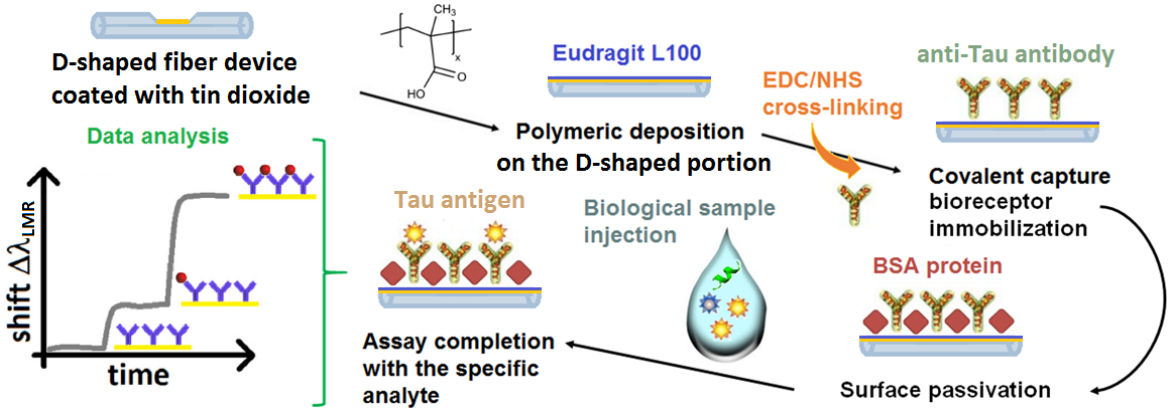

Figure 2. Schematic representation of the biolayer preparation on the sensitive region surface and of the antibody-antigen binding phase.

4. The optical experimental setup

In order to perform label-free biosensing measurements, an optical transmission setup was built up and mimicked the setups previously developed at CNR-IFAC.[9-11] Figure 3 details the setup used.

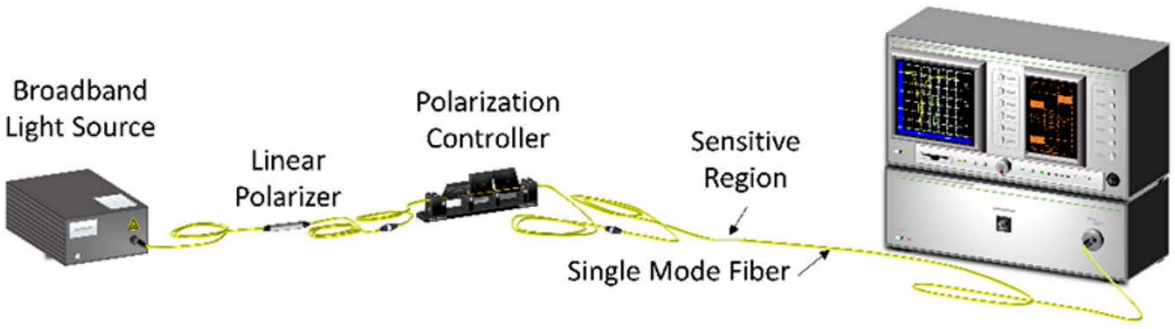

Figure 3. Optical setup used to perform the Tau protein experiment.

The setup consisted of a broadband multi-LED light source (FIBRELABS, Inc., SLD-1310/1430/1550/1690), an optical spectrum analyzer (OSA, Anritsu MS9030A-MS9701B) that made it possible to monitor a wavelength range from 1200 to 1700 nm, a fiber optic in-line polarizer and a polarization controller. The last two devices permitted exciting the D-shaped SMFs with a TE or a TM polarized signal.

5. Results and discussion

Preliminary results on the use of the anti-Tau/Tau protein pair are here detailed. In particular, the flow rate was reduced a lot (5 μL min^{-1}) with respect to previously experiments with different proteins (25 μL min^{-1}) given the lower affinity between the anti-Tau antibody and Tau antigen when compared with immunoglobulin G. This enlarged seriously the duration of the experiment up to 8 hours.

During the experiment, thanks to the optical setup used, it was possible to monitor in real-time the binding interactions for the SnO_2-coated LMR fiber sensors. Figure 4 details the signal change as a function of the time during the steps related to preparation of the biolayer.

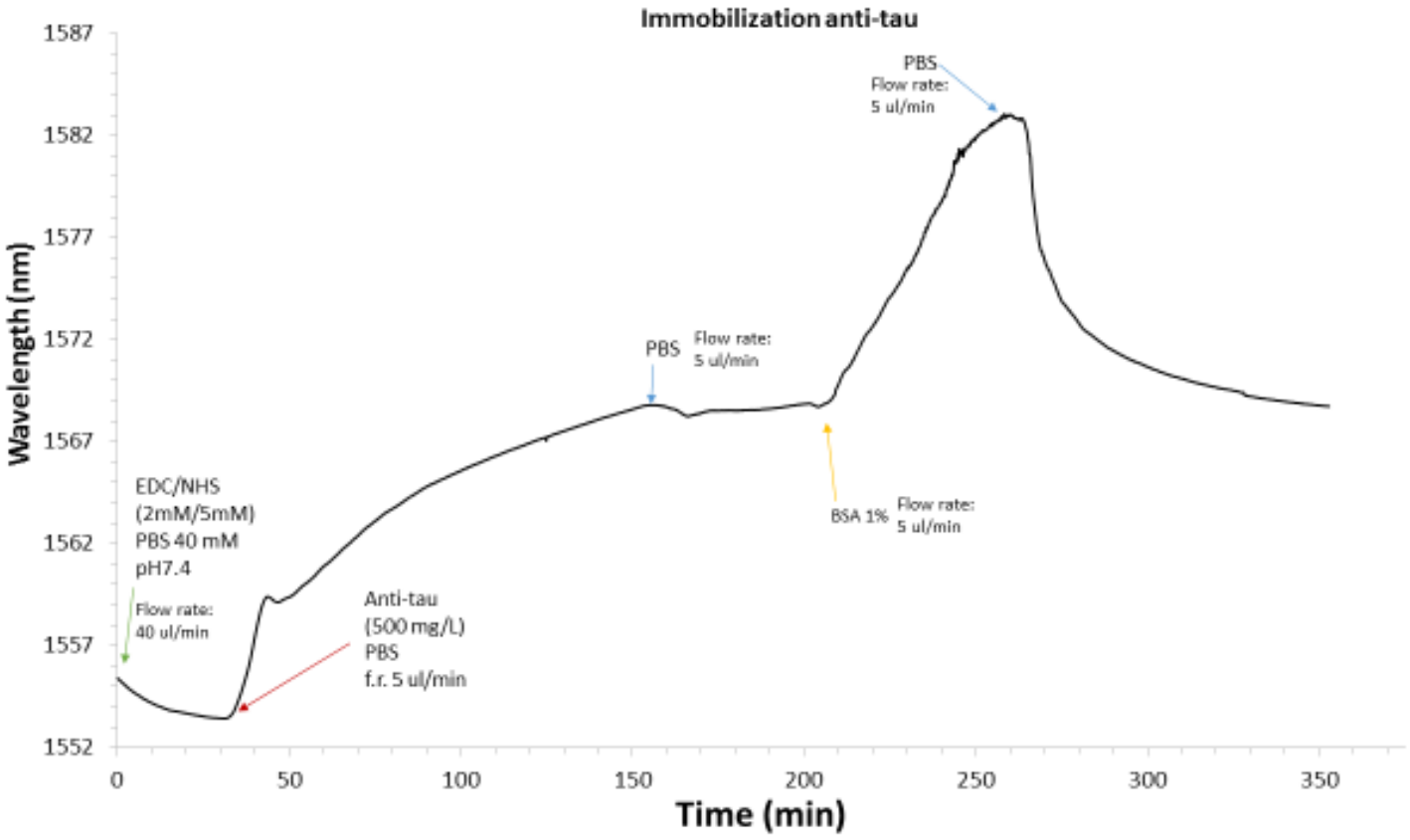

Figure 4. Sensorgram of the SnO2-coated D-shaped SMF biosensor starting from the activation of free functionalities, covalent anti-Tau antibody immobilization up to surface passivation with BSA.

Two main conclusions can be extrapolated:

1. the immobilization of the anti-Tau antibody (Tau441) follows always a double trend (fitted by a double exponential function) credibly related to an initial bound state with high mobility and a second unbound state with low mobility;

2. the passivation of the receptor layer with BSA seems not to be effective since, after the washing with PBS, the signal reaches the same value before the passivation. There could be another reason for that: no free functionalities are available to be blocked by BSA, which means that the surface coverage is total.

After the preparation of the biological sensing layer, increasing concentrations of the analyte (Tau46) spiked in a PBS matrix were injected inside the thermo-stabilized microfluidic system by means of a PC-controlled peristaltic pump.

The first experimental tests involved the injection of very low analyte concentrations, starting from 0.01 μg L^{-1} or 0.1 μg L^{-1}. It turned out that the signal slightly decreased after the injection of each concentration of analyte. This was in contrast with the previous results achieved with the same type of sensors coated with SnO_2 and tested as a function of increasing refractive index changes.[7,8] In fact, when there is a binding interaction between the deposited receptor and its high-affinity counterpart, the LMR signal is expected to increase (shift towards longer wavelengths) due to a surface density increase (increment in RI and thickness of the layer). This could be explained in different ways:

1. the passivation with BSA was not so good given the inherent characteristics of the TP, thus needing a different passivation molecule in order to guarantee a better fouling resistance of the biolayer;
2. the buffer we used had not the right pH or content, thus producing some changes in the anti-Tau biochemical features or structure;
3. the surface functionalization with Eudragit L100 copolymer was not so effective given the inherent characteristics of the TP, thus creating a negatively- or positively-charged surface. This reduces the attaching capability of the receptor.

Given the very first time that the Tau protein is used in combination with optical fiber sensors, we had evaluated the feasibility of the assay protocol always used in the past to characterize antibody-antigen binding interactions. Therefore, we increased the TP concentration up to 5 mg L^{-1} and even greater to assess the protocol proposed. Figure 5 details the sensorgram (signal change vs. time) of a SnO2-coated d-shaped SMF biosensor, starting from the injection of the negative control as the blank measurement followed by all the antigen concentrations. It can be observed that the signal was almost stable for the first TP injection (5 mg L^{-1}), but then it started to increase in the next two TP concentrations, with a tremendous shift obtained after the injection of 50 mg L^{-1}. The effect we saw could be ascribed to the great absorption of the TP with the sensing surface and not to the affinity attachment of the anti-Tau/TP pair.

We are totally sure that the LMR sensor worked properly, given the previous outstanding results attained with this sensing structure (see Publications).

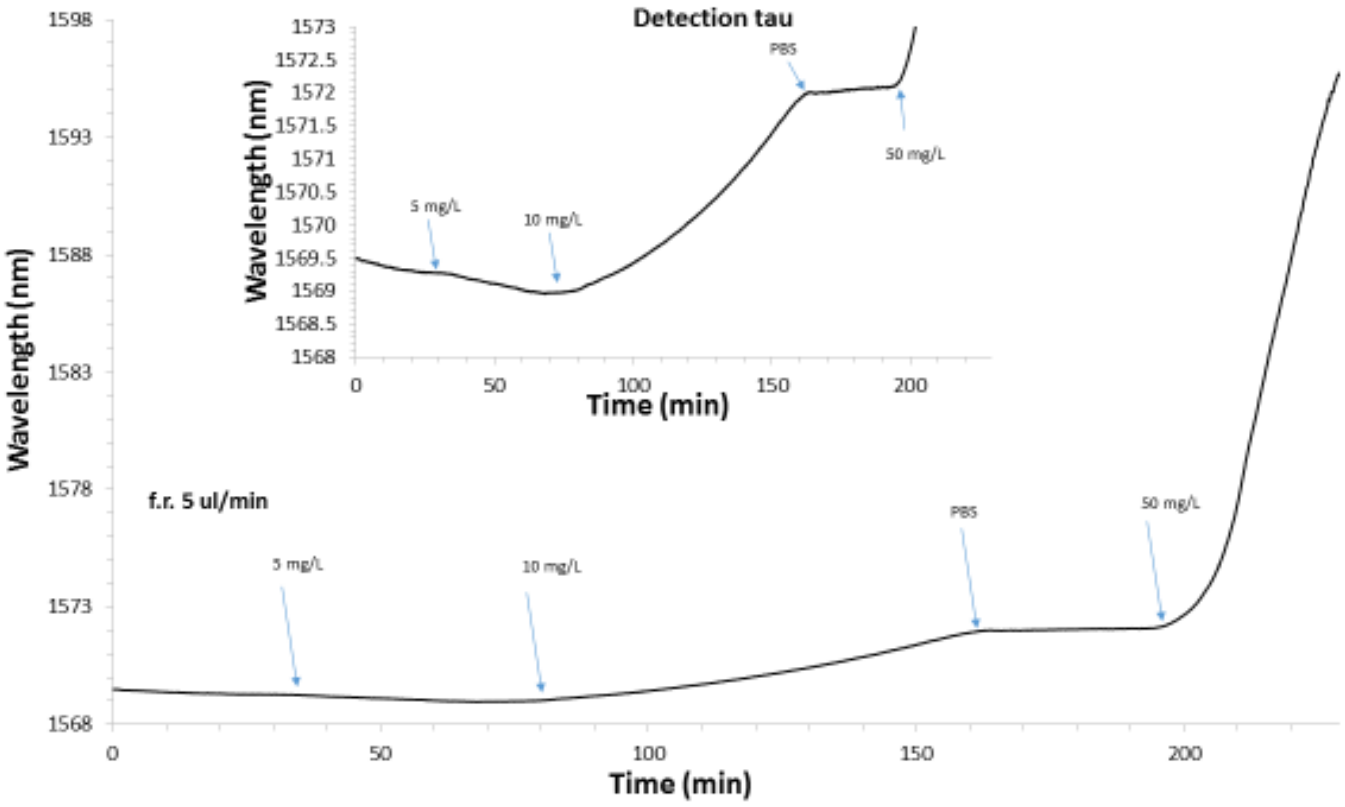

Figure 5. Sensorgram of the SnO_2-coated D-shaped SMF biosensor starting from the injection of the negative control to all the high concentrations of the Tau protein (5–50 mg L^{-1}).

6. Conclusions

The results expected by this Short Term Mobility program, were: the development and characterization of a special high-sensitivity fiber biosensor able to detect very-low changes in analyte concentrations, especially the detection of Tau protein one of the most important biomarker for Alzheimer's disease. Further aim of the stay would have been to consolidate the collaboration between the two institutes involved, between which future formal collaborations will be signed.

The main task was achieved with the design and characterization of a LMR D-shaped SMF biosensor coated with tin dioxide able to detect very low concentrations of immunoglobulin G (see Publications). We will further investigate the incredible fascinating topic here proposed, the detection of Tau protein at very low concentrations. In particular, we will test these two solutions:

1. reduction of the solution's volume by means of scaling down the flow cell size and of reducing the flow channel length. This will shorten the time of experiment and made faster the binding interactions;
2. use of different antifouling materials to better passivate the sensing surface, like ethylamine, aminoethoxy acetic acid and so on, in order to maintain an hydrophilic sensing surface.

The last, but not the least, the collaboration with the prestigious and growing Institute of Smart Cities, Public University of Navarra, was consolidated.

7. References

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Publications

The STM program, giving the possibility to start the collaboration between the two involved Institutes, will consolidate the existing collaboration in order to continue investigating the fascinating subject here proposed. Moreover, the STM program gave also the possibility to submit two papers already:

A. **Chiavaioli, F.**, Zubiate, P., Del Villar, I., Zamarreño, C.R., Giannetti, A., Tombelli, S., Trono, C., Arregui, F.J., Matias, I.R., Baldini, F. (under review to **ACS Sensors**). "Beyond the detection limit of optical biosensors with nanocoated fibres."

B. 4-pages abstract (entitled "Ultra-low detection limit lossy mode resonance-based fibre-optic biosensor") submitted to the **26th International Conference on Optical Fibre Sensors (OFS) 2018**, September 24-28, 2018, SwissTech Convention Center EPFL Campus, Lausanne, Switzerland.

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