MAP-Fis Joint Doctoral Programme

WORK PLAN

DEVELOPMENT OF NANOPLASMONIC THIN FILM BIOSENSORS WITH ENHANCED SENSITIVITY FOR DETECTION OF OCHRATOXIN-A

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SUMMARY

This project aims the development of nanoplasmonic materials, with enhanced sensitivity, for detection of a mycotoxin (Ochratoxin-A), commonly found in wine and grape juice. The detection will be based on the shift of absorption peaks of Au nanoparticles dispersed in TiO_2, resulting from the interaction between mytoxins/ nanoparticles (Localized Surface Plasmon Resonance phenomenon). A combination of environmentally friendly and cost-effective physical Glancing Angle Deposition approach will be used to prepare the films, allowing the preparation of specific architectures (e.g inclined columns, spirals, etc.) that may retain better and easily the toxins (e.g. by tailoring density, porosity) for their recognition and quantification. To tailor the metal nanoparticles morphology (size, shape and distribution), thermal annealing treatments and plasma activation routines will be optimized. After a proper functionalization with biorecognition elements, the sensor prototypes will be integrated in a laboratory-sized optical system to monitor the LSPR band changes, in the presence of Ochratoxin-A.

1. State of art

Ochratoxin-A (OTA) is a mycotoxin that occurs naturally and can contaminate crops in the field or after harvest. Grains used for human nutrition (rice, peanut, coffee, cereals) and food derivatives as wine, are the main sources of OTA [1]. The ingestion of OTA even in very small concentrations, causes progressive tissue damage in some organs of the human body (e.g. Liver, Kidneys), being also carcinogen [2,3]. Maximum permitted levels of OTA are established by EU legislation: 2 μg/kg for wine and grape juice (IP/04/1215 and Regulation 466/2011).

Nowadays, when dealing with biomolecule detection, the golden standard is the enzyme-linked immunosorbent assay (ELISA) [4], although other solutions are available (e.g. immunoaffinity chromatography [5] and fluorescence-based assays [6]). Nevertheless, in general, these methods are time-consuming, need external labels, and usually need well-trained handlers. Therefore, the development of alternatives based on novel lab-on-chip biosensing technologies has exponentially increased in the last years [7,8].

In this context, biosensors based on thin films arise as sensitive, specific, easy to handle, with rapid response time and cost-effective. Of great interest are those based on gold (Au) thin films, taking profit of the phenomenon of surface plasmon resonance (SPR) [9]. More recently, nanoparticles are also gaining huge relevance, due to the confined nature of localized surface plasmons (LSPs) [10–12]. A major advantage is the fact that LSPs can be excited by the passing-through light, offering the opportunity to simplify the optical detection systems [13], outmatching SPR biosensing in terms of fabrication and experimental setup costs [10,12]. The detection mechanism in LSPR sensing is based on the interaction between the nanoparticles and (bio)molecular species of interest, which leads to detectable shifts in the LSPR band. However, in practice, some obstacles related to the nanostructure of these materials, as well as the low active surface area, have hindered the application of this promising concept [14].

Plasmonic thin films consisting of Au nanoparticles, dispersed in oxide matrixes, are being extensively studied in the group led by Filipe Vaz in the past years [15–20]. Magnetron sputtering is widely considered to be versatile and cost-effective technique to prepare such thin films. Combined with a post-deposition annealing treatment, it allows to tune LSPR absorption bands, by changing the concentration of plasmonic

metal, the host matrix material, and the size distribution of nanoparticles [17,20]. To further tailor the microstructure/ porosity of the films [21–24], it was implemented a GLancing Angle Deposition (GLAD) system. In a recent work (2019) [25], it was showed that the GLAD approach allows to improve the sensitivity of the films to gas molecules by enhancing adsorption sites.

Regarding biosensing, the past months were dedicated to the first studies related to the immobilization of (bio)molecules (e.g. antibodies, proteins) onto the surface Au-TiO_2 thin films [26]. Nevertheless, the adsorption area was considered insufficient to obtain a high-sensitive LSPR biosensor. Yet, there is a large margin for improvement, which means that the development of sensitive LSPR biosensors (e.g. to detect a specific mycotoxin as OTA) is an attainable goal [26].

2. Objetives

To enhance the performance of LSPR biosensors, it is proposed the production of plasmonic nanocomposite thin films with tailored architectures by GLancing Angle Deposition (GLAD). The developed plasmonic thin films will serve as platforms to detect a specific mycotoxin, namely Ochratoxin-A (OTA), which can be found in grape juice, take in by vulnerable consumers like children, as well as in wine. The sensor prototypes will be integrated in a laboratory-sized optical system to monitor the targeted biomolecule (OTA).

Therefore, the mail goal of this project is the development of high-sensitive plasmonic materials, based on porous nanostructured oxide thin films containing gold nanoparticles, functionalized with specific bio-recognition elements, to detect low concentrations of OTA.

To achieve the main goal, different objectives must be accomplished:

1. Preparation of nanoplasmonic thin films, composed by Au nanoparticles dispersed in TiO_2, using the technique of magnetron sputtering followed by post-deposition treatments;
2. Improve of the sensitivity of the thin films, by enhancing adsorption sites through the preparation of porous oxide matrixes by GLancing Angle Deposition (GLAD);
3. Promote the selectivity of the nanoplasmonic thin films through the immobilization of (bio)molecules with enhanced adsorption area;
4. Development of a laboratory-sized optical sensing system with fast response, integrating the LSPR biosensors and a microfluidic platform;
5. Demonstration of the applicability of this technology, and validate the efficiency, sensitivity and selectivity of the LSPR sensors at the laboratory scale.

The objectives of this PhD project proposal also fall within the scope of a FCT funded project NANO4BIO (PTDC/FIS-MAC/32299/2017), which aims the development of an optical biodetection system, able to detect low concentrations of OTA. In the frame of this funded project, different deposition approaches run in parallel to optimize nanoplasmonic films (e.g. using a cluster gun and ion implantation), but all converging to the same application: LSPR sensing.

3. Detailed Description

This project aims to contribute for the Regional Strategy of Smart Specialization in two domains, namely the Wine Industry and Advanced Production Systems. The Wine Industry represents one of the most important products for the local economy, and it is of paramount importance the development of fast diagnostic systems/sensors to attest its quality. Nanotechnologies are also highlighted, since they are considered an emerging scientific area due to great investments that have been applied in infrastructures, research programs and technological development in the past few years. The application of Advanced Technologies to Materials on Health is also an important area of actuation.

It is aimed the development of LSPR biosensors for an early detection of mycotoxins, e.g. in grape juice and wine, which is expected to prevent diseases caused by their ingestion, contributing to the "ensurance of healthy lives and promote well-being" (GOAL3). Another innovation brought by this project is the use of a new type of deposition system to enhance the sensitivity of biosensors. GLancing Angle Deposition (GLAD) to prepare such thin films is an attractive strategy to obtain a wide variety of nanostructures (inclined columns, zigzag and spirals), originating different physical responses [21–24]. Using recognized and clean deposition techniques, this work is also in compliance with GOAL17. These technologies can be easily upscaled at the industrial level, "promoting inclusive and sustainable industrialization and foster innovation", aligned with GOAL9 objectives.

This project is planned to be developed in 4 years, culminating with a doctoral thesis. Here are the Tasks to accomplish the proposed objectives (timeline attachment):

Task 1. Bibliographic review

An extensive bibliographic review of the state-of-the-art of the related topics will be continuously performed.

Task 2. Production of nanoplasmonic thin films

T2.1 – Deposition of Au-TiO_2 by reactive magnetron sputtering (CF-UM-UP)

Nanocomposite thin films composed of Au seeds dispersed in an oxide matrix (TiO_2) will be deposited by reactive DC magnetron sputtering onto silicon, for characterization purposes, and glass/quartz substrates to prepare the sensor . A metallic Ti target, with

different amounts of Au pellets placed on its erosion zone will be sputtered, in a reactive atmosphere ($Ar+O_2$) [17].

T2.2 – Deposition of architectured thin films (CF-UM-UP)

The Glancing Angle Deposition technique relies on a non-stationary substrate holder that allows two basic types of movements (α and ϕ rotation,), in order to achieve the desired film architectures, namely i) inclined and zigzag columns; ii) spirals; iii) isolated columns. The GLAD system was recently installed in the research group [25].

Task 2.3 – Growth of the nanoparticles by post-deposition treatments (CF-UM-UP)

Since the average size of the Au seeds are typically lower than 10 nm [15], a post-deposition treatment is paramount to promote the growth of the nanoparticles and thus to obtain the desired LSPR bands. In order to turn the growth of nanoparticles more efficient, an alternative approach to the thermal annealing protocol will be tested, namely using plasma treatments.

Task 3. Characterization of the thin films

T3.1 – Composition, structure and morphology of the films (3B's)

The micro/nano-structure (morphology, composition, nanoparticles' size distribution, shape and volume fraction) of the films will be probed directly by high-resolution scanning, and transmission, electron microscopy (HR-SEM/TEM) with Energy Dispersive X-Ray Spectroscopy (EDX).The structure of the thin film will be studied by X-Ray Diffraction (XRD).

T3.2 Surface analysis (3B's)

Since these films are expected to be a Transmittance-LSPR platform for sensing, the chemistry and physical properties of their surfaces must be extensively studied. Therefore, surface topography, roughness and surface chemistry of the nanoplasmonic films will be analysed in detail by different techniques, such as Atomic Force Microscopy (AFM) and X-ray photoelectron spectroscopy (XPS).

Task 4: Preparation of the optical sensing system

T4.1 Optical response of the films in the transmission mode (CF-UM-UP)

The Transmission-LSPR bands of the films will be measured and correlated with the structure and morphology of the nanocomposites.

T4.2 Fitting the LSPR band (CF-UM-UP)

A polynomial fit will be employed for an easy and fast analysis of the main characteristics of the LSPR band changes (peak position, width and asymmetry) caused by different environments. The algorithm gives consistent results [25], and can be adapted to different plasmonic systems.

T4.3 Preparation of microfluidic channels (CF-UM-UP and 3B's)

The optical sensing system of the group (LSPR gas sensing) [25] will be adapted to include a transparent microfluidic platform, polydimethylsiloxane (PDMS), to pump analyte solutions to the surface of the LSPR biosensor. The "sensing chips" are obtained by sticking the LSPR biosensor to PDMS using O_2 plasma treatment [27].

Task 5: Functionalization of the nanoplasmonic thin films

T5.1: Surface activation (CF-UM-UP)

The surface of the films will be activated by plasma: I) argon plasma allows to partially expose the Au nanoparticles, thereby creating nanoparticles' adsorption sites- this improves the sensitivity of the thin film [17]; II) Oxygen plasma are used to create functional groups at the film's surface.

T5.2: Immobilization of biorecognition elements (3B's)

The selectivity of the thin films will be reached through immobilization of biorecognition elements directly on the nanoparticles, namely using antibodies (e.g. anti-OTA) and specific blocking proteins (e.g. BSA) [14]. To enhance chemical binding sites, self-assembled monolayers and other linkers (-thiol, -amine functional groups) will be tested [28].

Task 6. Prototype testing

T6.1 Refractive index sensitivity tests (CF-UM-UP)

The LSPR biosensors prototypes will be tested in the optical sensing system. The refractive index sensitivity (RIS) of the films will be estimated using liquids with different refractive indexes [22].

T6.2 Detection of the specific biomolecule – Ochratoxin-A (CF-UM-UP)

The LSPR biosensors will be tested against OTA solutions, with different concentrations, to establish the limit-of-detection (LOD) and the signal-to-noise-ratio (SNR) of the system, which are expected to be competitive against conventional techniques, namely ELISA. Finally, specificity of these sensors will be validated using "real" samples (wine and grape juice).

Task 7. Dissemination and exploitation

Reports and publications

A significant focus will be assigned to the submission of high-quality research papers to peer-reviewed journals, and attendance of national and international conferences. Progress reports will be written periodically.

Task 8. Doctoral Thesis

4. References

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