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Biological behaviour of thin films consisting of Au nanoparticles dispersed in a TiO_2 dielectric matrix

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ABSTRACT

In this work it was studied the possible use of thin films, composed of Au nanoparticles (NPs) embedded in a TiO_2 matrix, in biological applications, by evaluating their interaction with a well-known protein, Bovine Serum Albumin (BSA), as well as with microbial cells (*Candida albicans*). The films were produced by one-step reactive DC magnetron sputtering followed by heat-treatment. The samples revealed a composition of 8.3 at.% of Au and a stoichiometric TiO_2 matrix. The annealing promoted grain size increase of the Au NPs from 3 nm (at 300 °C) to 7 nm (at 500 °C) and a progressive crystallization of the TiO_2 matrix to anatase. A broad localized surface plasmon resonance (LSPR) absorption band ($\lambda = 580-720$ nm) was clearly observed in the sample annealed at 500 °C, being less intense at 300 °C. The biological tests indicated that the BSA adhesion is dependent on surface nanostructure morphology, which in turn depends on the annealing temperature that changed the roughness and wettability of the films. The Au:TiO_2 thin films also induced a significant change of the microbial cell membrane integrity, and ultimately the cell viability, which in turn affected the adhesion on its surface. The microstructural changes (structure, grain size and surface morphology) of the Au:TiO_2 films promoted by heat-treatment shaped the amount of BSA adhered and affected cell viability.

1. Introduction

Nanocomposite thin films containing noble metal nanoparticles (NPs), embedded in a host dielectric matrix, have attracted the interest of scientists in both basic scientific research and in a wide range of technological applications. Beyond those of purely decorative purposes, as observed in the windows of the medieval cathedrals and in Roman glasses (such as the well-known Lycurgus Cup from the 4th century [1,2]), plasmonic materials can be found in many applications. Some examples are in the field of photovoltaics, pollutant-degradation materials, gas sensors, Surface-Enhanced Raman Scattering (SERS), photochromism, chemical surface activation, optical tweezers, among several others [2–12]. Systems composed of noble metals (e.g. Au, Ag) and oxides (e.g. TiO_2) have been also extensively studied due to their functional properties for antibacterial applications [13] and in the study of biological processes in live cells [14]. Moreover, the knowledge acquired about plasmonic materials allowed building chemical and biological sensors [15], which are based on the detection of changes

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of their optical response due to presence of molecules and biolayers. Nowadays, the area of optical biosensors, namely surface plasmon resonance (SPR) biosensors, is very successful in a wide range of fields, from fundamental biological studies to the detection of chemical and biological species in different areas such as food quality and safety analysis (e.g. pathogens, toxins), medical diagnostics (e.g. drugs) and environmental monitoring (e.g. pesticides, heavy elements) [16–18].

Recently, it has been realised that the sensor transduction mechanism of the localised (L)SPR-based nanosensors is analogous to that of SPR biosensors, which rely on gold-based sensors [19]. In LSPR sensors the optical response strongly depends on the clustering tendency of the nanoparticles, namely the clusters size, shape and distribution, the interaction between them, as well as on the host matrix dielectric function itself [11,20–22].

In this work, Au:TiO_2 thin films were produced by one-step magnetron sputtering deposition and post-deposition annealing (in order to promote the Au nanoparticles formation). The microstructure of the films (characterized in terms of crystalline structure, grain size, phase composition and surface morphology) was firstly analysed and correlated with the optical responses of the films, as a function of the annealing temperature. Afterwards, and taking into account envisaged applications using these plasmonic nanocomposite materials (for biosensing devices and antimicrobial surfaces), the interaction of the films with a well-known protein, Bovine Serum Albumin (BSA), as well as with microbial cells (*Candida albicans*) were analysed. On the one hand, the BSA was selected since protein adsorption is the first of a complex series of events that regulates many phenomena at the nano-biointerface [23] and thus the understanding of how the nanocomposite (micros)structure influences protein adsorption is fundamental for many processes such as those related with affinity biosensors [17]. On the other hand, the films were also tested in terms of antimicrobial activity. Since TiO_2 thin films do not present antimicrobial activity against *C. albicans* without UV irradiation or without doping the surface with an antimicrobial agent (e.g. Ag [24]), the influence of the embedded Au nanoparticles on the TiO_2 host matrix was also evaluated.

2. Experimental details

2.1. Au:TiO_2 thin films preparation and characterization

The Au:TiO_2 thin films were deposited on glass lamellae ISO 8037 (for the optical characterization, surface morphology analysis and biological-related tests) and silicon <100> (for chemical, structural and morphological analysis) substrates using reactive DC magnetron sputtering. One-step deposition has been used to deposit the host matrix (TiO_2) with embedded gold atoms/clusters. The cathode was composed of a titanium target (99.6% purity), with three pellets of Au (each one with 15 mm^2 of surface area), placed on the preferential erosion zone of the target. A DC current of 2 A was applied. The substrates were placed on a rotating sample holder, positioned at 70 mm from the target, with a constant rotation speed of 9 rpm, heated at 100 °C. A mixture of argon and oxygen with a constant flow (60 sccm and 6 sccm, respectively) was injected into the deposition chamber and the working pressure was kept approximately constant for the deposition process at about 0.5 Pa. After 90 min deposition, the samples were subjected to an in-air annealing procedure at several temperatures, ranging from 200 to 800 °C. The heat-treatment experiments were conducted in a furnace at atmospheric pressure. The annealed temperatures chosen were reached rising the temperature at a rate of 5 °C/min until the desired temperature was attained, fixing this temperature for 60 min (isothermal period). The samples cooled down freely before their removal from the furnace.

The chemical composition of the films and uniformity across thickness was investigated by Rutherford Backscattering Spectrometry (RBS). RBS measurements were carried out with 2 MeV ^4He at an angle of incidence 0° in the small (RBS) chamber. There were three detectors in the chamber: standard at 140°, and two pin-diode detectors located symmetrical each other, both at 165° (detector 3 on same side as standard detector 2). The RBS data were analysed with the IBA DataFurnace NDF v9.6a [25]. The crystalline structure of the Au:TiO_2 thin films was studied by *in-situ* X-ray Diffraction (XRD) during annealing, using a θ-θ Brucker D8 Advance System diffractometer, with a Cu-Kα radiation, in a Bragg-Brentano configuration, equipped with a furnace and applying the same annealing procedure as described above. The Au nanoparticles size was determined by fitting the Au diffraction peaks with a Pearson VII function, using the winfit software [26]. The morphology and thickness of the samples annealed at different annealing temperatures were studied using a FEC-SEM Nova 200 NanoSEM scanning microscopy, in cross-sections of the Au:TiO_2 films. The film surface topography was analyzed by Atomic Force Microscopy, using a MultiMode SPM microscope, controlled by a NanoScope IIIa from Digital Instruments, in order to characterize the effect of the annealing in the surface roughness. Further analysis of the surface of the Au:TiO_2 films was conducted by measuring its contact angle with 3 μL of water, using a OCA 20 angle meter from Dataphysics. The optical response of the films (transmittance) were characterized in the spectral range between 250 and 1800 nm using a Shimadzu UV-3101 PC UV–vis–NIR spectrophotometer.

2.2. Adhesion of proteins, Bovine Serum Albumin (BSA), to the Au:TiO_2 films

The adhesion properties of Au:TiO_2 thin films annealed at different temperatures were evaluated by incubating with a solution of Bovine Serum Albumin (BSA). After washing the films, with ethanol and deionised water, they were incubated with a 3 mM aqueous solution of BSA for 24 h at a constant stirring of 80 rpm. After the incubation, a part of the BSA solution was stored at −20 °C, for posterior analysis, and the thin film was used for optical analysis. The protein that remained in the solution was quantified by using Bradford's protein assay [27], and the integrity of the proteins analysed by a 12% SDS-polyacrylamide gel electrophoresis (SDS-PAGE) [28]. After the electrophoresis, the gel was washed and stained with Brilliant Comassie Blue.

2.3. Adhesion of microorganisms (Candica albicans), to the Au:TiO_2 films

To further study the adhesion behaviour of the samples, the Au:TiO_2 nanocomposite thin films were incubated with yeast cells. The yeast *C. albicans* (strain SC5314) was obtained from the Centre of Molecular and Environmental Biology (CBMA), Department of Biology, University of Minho. A pre-culture was prepared for each individual batch experiment. One colony of *C. albicans* strain was picked and loop inoculated into a 125 ml Erlenmeyer flask, containing 20 mL of Yeast Peptone Dextrose growth medium (1% bactopeptone (w/v), 1% yeast extract (w/v), 2% Glucose (w/v)), and incubated at 30 °C, for 12–15 h. On the next day, the cells were transferred into different 250 ml Erlenmeyer flasks containing 50 ml of YPD broth medium at an initial optical density (OD) of 0.1 measured at a wavelength of 600 nm. The thin films (a square of 26 × 26 mm^2 deposited on glass), previously sterilized with 70% ethanol for 1 h and rinsed in sterile water, were placed on the bottom of the flask. Flasks were then shaken at 80 rpm at 30 °C, and