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A comparative study of fibrinogen adsorption onto metal oxide thin films

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ABSTRACT

One of the first events occurring upon foreign material-biological medium contact is the adsorption of proteins, which evolution greatly determines the cells response to the material. Protein–surface interactions are a complex phenomenon driven by the physicochemical properties of the surface, protein(s) and liquid medium involve in the interaction. In this article the adsorption of fibrinogen (Fbg) onto Ta_2O_5, Nb_2O_5, TiO_2 and ZrO_2 thin films is reported. The adsorption kinetics and characteristics of the adsorbed fibrinogen layer were studied in situ using dynamic and spectroscopic ellipsometry. The films wettability, surface energy ($\gamma^{LW/AB}$) and roughness were characterized aiming to elucidate their correlations with Fbg adsorption. The adsorption rate changed accordingly to the film; the fastest adsorption rate and highest Fbg surface mass concentration (Γ) was observed on ZrO_2. The hydrophobic/hydrophilic character of the oxide highly influenced Fbg adsorption. On Ta_2O_5, Nb_2O_5 and TiO_2, which were either hydrophilic or in the breaking-point between hydrophilicity and hydrophobicity, Γ was correlated to the polar component of $\gamma^{LW/AB}$ and roughness of the surface. On ZrO_2, clearly hydrophobic, Γ increased significantly off the correlation observed for the other films. The results indicated different adsorption dynamics and orientations of the Fbg molecules dependent on the surface hydrophobic/hydrophilic character.

1. Introduction

A biomaterial has to fulfill different requirements in order to be of practical use, many of which depend on its functional application and are mostly related to the bulk properties of the material. On the other hand, a crucial requirement to any biomaterial is the biocompatibility, which is mainly related to the surface properties of the material [1,2]. Therefore, an interesting approach to produce biomaterials by design could be divided into two steps; fulfillment of the functional requirements through tailoring of the bulk properties and tuning of the interaction with the biological medium through surface modifications, such as the use of appropriate coatings.

This approach demands a deeper knowledge of the phenomena governing the interactions between foreign materials and the biological ambiance in order to minimize the trial and error attempts during the coatings development. It has been well established that whenever a foreign material comes in contact with physiological or extracellular matrix fluids, a spontaneous adsorption of water molecules and solvated ions occurs on its surface and immediately after, proteins are adsorbed on the surface [1–4]. The profile of the adsorbed protein layer, which in turn drives the cellular interaction with the material, highly depends on the physicochemical properties of the surface [5,6]. Thus, a fundamental understanding of the effect of the physicochemical surface properties on the protein adsorption phenomenon is crucial to understand the biocompatibility principles [7–9].

This is the motivation to study the protein adsorption on a specific family of coatings, which are proposed as a surface modification to improve the biocompatibility of materials such as stainless steel (SS), which possess adequate bulk properties to develop SS-based biomaterials for orthopedic or dental implants and is less expensive than the widely used Ti; but has a poorer biocompatibility compared to that of Ti.

Materials based on metals such as Ta, Nb, Zr or Ti, which outer surface layer normally consists of their native oxides, have been proved to allow and promote cell adhesion and proliferation [4,10,11]. Moreover, these oxides have shown acceptable bio-corrosion resistance and mechanical properties, constituting promising materials for the development of biocompatible metal oxide coatings [12–14]. Although the recognized biocompatibility of these oxides, the basis of protein adsorption on them has not been extensively studied. In particular, for fibrinogen (Fbg) adsorption few studies have been carried out on these metal oxides, either

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on native or deposited oxides; apart from those performed on the more commonly studied TiO_2 [15–21]. Furthermore, normally, the correlations between surface physicochemical properties and protein adsorption found for a specific family of materials, such as the polymers, cannot be a priori extended to a different family of materials, such as the metal oxides. At the same time, the different physicochemical characteristics of different proteins imply that the behavior of a protein upon adsorption on certain surface may differ from the one of a different protein upon adsorption on the same surface. This remarks the need for a further study of the adsorption of different proteins on different metal oxides.

In this research, we proposed Ta_2O_5, Nb_2O_5, ZrO_2 and TiO_2 thin films as model surfaces to study the correlations between surface properties and protein adsorption, aiming to contribute to the acquisition of a deeper and basic understanding of the principles of their biocompatibility and the correlations between the surface physicochemical properties of metal oxides and protein adsorption. The adsorption of Fbg is one of the initial events occurring during the blood-material interaction and is crucial for hemocompatibility tests; therefore, it was chosen as the model protein in the present work [22]. Fibrinogen is a soluble plasma glycoprotein highly involved in the activation of the coagulation cascade and the response of the immune system, promoting cell adhesion and having an active role in the inflammation processes [22–24].

In this paper, the results of Fbg adsorption on Ta_2O_5, Nb_2O_5, ZrO_2 and TiO_2 thin films deposited onto Si(1 0 0) wafers by radio frequency (RF) magnetron sputtering are reported; as part of a more extensive research, involving the study of the adsorption of different proteins on well-characterized homogeneous metal oxide thin films [25]. The surface energy, wettability, chemical composition, structure and optical properties of the films were obtained in order to explore the correlations between the surface properties and the Fbg adsorption. The adsorption of Fbg was studied in situ at the solid-liquid interface using ellipsometry; a non-destructive optical technique widely used to study the adsorption of thin organic layers on reflective solid surfaces [26–28]. Dynamic ellipsometry (DE) at fixed photon energy was used to monitor the kinetics of the Fbg adsorption, while spectroscopic ellipsometry (SE) was used to characterize the thickness and density of the adsorbed Fbg layers.

2. Experimental details

2.1. Oxide film deposition and characterization

Thin films of Ta_2O_5, Nb_2O_5, ZrO_2 and TiO_2 were deposited onto Si(1 0 0) wafers by reactive RF Balanced Magnetron Sputtering from metal targets, Ti, Ta, Nb or Zr (99.95% purity), under the same deposition conditions; atmosphere gas composition of 80% Ar and 20% O_2, deposition pressure of 4 Pa, RF power of 200 W and 1800 s of deposition time.

The chemical composition of the films was characterized using X-ray photoelectron spectroscopy (XPS), in a VG Microtech Multilab ESCA 2000 using Al Kα ($h\nu$ = 1486.6 eV) non-monochromatic radiation, a 500 μm spatial resolution and 20 eV pass energy for the acquisition of the high resolution spectra. No cleaning process, such as Ar ion bombardment, was performed on the films in order to obtain the surface composition of the films in the same state as they interacted with the fibrinogen. The high resolution spectra obtained in the C 1s, O 1s and Ta 4d, Nb 3d, Ti 2p or Zr 3d photoelectron peaks energy regions were fitted using the SDPv4.1 software® to obtain the films elemental composition. The structure of the films was explored using a Bruker D8 XRD system in the Bragg–Bretano mode and monochromatized Cu Kα radiation. The root mean square roughness (RMS) of the films was obtained using a Profilometer DEKTAK II, scanning 250 μm and averaging 20 different scans.

The surface energy and wettability properties of the films were obtained by contact angle (CA) measurements performed in static sessile drop mode in a Ramé-Hart Inc. Goniometer and using uniform 4 μL drops of the probe liquids; double distilled water, formamide, diiodomethane and dimethyl sulfoxide. The profile of the droplet and the CA were determined using the Drop Snake software [29]. The surface energy calculations were performed following the van Oss, Good and Chaudhury method [30].

Optical characterization of the films was done using a Jobin Yvon Uvisel (JYU) DH10 ellipsometer; the same equipment used to perform all the ellipsometric measurements throughout the present study. Ellipsometric spectra of the films were acquired from 1.5 to 5 eV at an angle of incidence (AOI) of 70° with the films inside an empty JYU liquid cell to include any possible effect of the cell windows. The spectra were fitted to a four-phase optical model (Fig. 1(a)), using the DeltaPsi2 software® to obtain the films optical properties, which were later used to model the adsorbed Fbg layers.

2.2. Preparation of the Fbg solution

A fresh stock of 0.5 mg/mL Fbg solution was prepared before each experiment by dissolving the required quantity of lyophilized Human Type I Fibrinogen (Sigma-Aldrich, F3879) in phosphate buffer saline (PBS) 0.01 M, pH 7.4. A 15 mL aliquot of the solution was placed into the JYU liquid cell to acquire an ellipsometric spectrum from 1.5 to 5 eV at an AOI of 60°, which guaranteed light reflection only from the surface of the solution. The spectrum was fitted to a two phase optical model (Fig. 1(b)), using the DeltaPsi2 software®, to obtain the optical properties of the solution.

2.3. Adsorption of Fbg

Each oxide film was immersed into 13 mL of freshly prepared Fbg solution in the JYU liquid cell. Immediately after immersion, a DE routine was started to obtain the ellipsometric angles, Ψ and Δ, every 2 s at an AOI of 70° and a fixed energy of 3 eV. After 2400 s of immersion, an ellipsometric spectrum from 1.5 to 4.2 eV was then acquired in situ. The spectrum was fitted to a four phase optical model (Fig. 1(c)), to obtain the dispersion relation of the refractive index, n_p, and the thickness, d_p, of the adsorbed Fbg layer. In the model, the optical phase representing the adsorbed Fbg layer was parameterized using a transparent Cauchy function. The optical properties corresponding to the substrate and the liquid-ambient were fixed to the previously obtained functions of the oxide film and the Fbg solution, respectively. The χ^2 value was the figure of merit to evaluate the quality of the SE data fitting.

The n_p @ 2 eV and the d_p values obtained were used to calculate the surface mass concentration of the adsorbed Fbg layer, Γ, according to the De Feijter's formula [31], Eq. (1).

$$\Gamma = \frac{d_p(n_p - n_m)}{\partial n/\partial C} \quad (1)$$

where $\partial n/\partial C$ corresponds to the refractive index increment with protein concentration and n_m to the refractive index of the Fbg solution @ 2 eV. For $\partial n/\partial C$ a value of 0.18 cm^3/g was used [32–34].

To ensure reproducibility of the results, four adsorption experiments were performed for each different oxide film; the reported values correspond to the arithmetic mean of the results and the error associated to them corresponds to the standard deviation of the four experiments.