Abstract

During this research thesis, protein adsorption phenomenon of basic blood plasma proteins on amorphous hydrogenated carbon (a-C:H), amorphous boron nitride (a-BN) and titanium diboride (TiB_2) thin films was studied. Polymeric films contanting zinc oxide (ZnO) nanoparticles were developed and their antibacterial properties against the species *Staphylococcus aureus* was investigated. The antibacterial properties of a-C:H and TiB_2 was also studied. Spectroscopic Ellipsometry (SE) was implemented for the study of the optical properties of the thin films and the adsorbed proteins, as well as for the real-time monitoring of protein adsorption. The surface topography and the thin films and the adsorbed proteins was studied by Atomic Force Microscopy (AFM) technique. Electric Force Microscopy (EFM) and Contact angle (CA) techniques were used for the study of surface electric charge distribution and of the wettability properties.

The main results of this research thesis are resumed below:

In the case of a preliminary assessment of the haemocompatibility of the thin films through the study of the adsorption of human serum albumin (HSA) and fibrinogen (Fib), the properties of the thin films were correlated with the ratio of HSA / Fib adsorption (Γ_{HSA} / Γ_{Fib}). The surface roughness of a-C:H thin films plays a crucial role, since the rougher samples favour the adsorption of HSA (their surface roughness is comparable to the HSA molecule size); thus, they present higher Γ_{HSA} / Γ_{Fib} value. The Γ_{HSA} / Γ_{Fib} values concerning boron-containing thin films (a-BN and TiB_2) are higher than these of a-C:H films. Their surface properties are important for the protein adsorption evolution. a-BN thin films containing more boron on their surface (less negatively charged surface) are also the most hydrophilic, and attract less the fibrinogen molecules, not favouring their adsorption. For the TiB_2 films, those containing more boron on their surface (more negatively charged surface) exhibit the higher Γ_{HSA} / Γ_{Fib} values.

The two proteins present similar optical properties. Both HSA and Fib are transparent until about 4eV, and albumin presents a slightly higher value of the refractive index ($n(\omega=0eV)=1.43$) that Fib's ($n(\omega=0eV)=1.40$). The energy of maximum optical absorption is around 7eV, mainly due the peptide bonds.

AFM topography images show that the adsorbed fibrinogen can either preserve its characteristic trinodular shape or have an oval shape. It can also have a linear or curved conformation, while many times the molecules seem to be conjunct.

The adsorption mechanisms of fibrinogen on a-C:H thin films were studied in real-time by the use of SE technique, for pH 7.4 and Fib's isoelectric point pI 5.8. The developed ellipsometric model described in detail the protein adsorption phenomenon, providing information about the protein layer thickness and the transformation of the protein molecules from the solution form to the adsorbed form. The fundamental gap

value (E_g) of the adsorbed fibrinogen indicates the molecules' dehydration. E_g is higher for the more hydrophobic a-C:H film, revealing that Fib is more dehydrated when adsorbed on it. The maximum optical absorption is exhibited at 5.55eV and 6.73eV for pH 7.4, and is due to the peptide bonds and the secondary structure of the protein. At pI 5.8, the contribution of the secondary structure no longer exists, while the contribution of aromatic side chains remains, thus shifting the energy E_o of maximum optical absorption at lower values.

The thickness and volume fraction of adsorbed fibrinogen are affected by the surface topography (nanostructure) and the wetting properties of the studied a-C:H thin films. The Fib adsorption is not favoured on the film with small nanostructures. Also, its hydrophobicity compels the Fib molecule to change conformation, with its hydrophilic groups in the interior of the molecule and its hydrophobic groups exposed to the surface of the thin film. This is the reason for the change / fluctuation of the thickness values and the volume fraction of the adsorbed Fib during its adsorption. On the other hand, a constant increase of these values is observed on the more hydrophilic film with larger surface nanostructures.

SE experimental results combined with an appropriate theoretical kinetic model revealed that the total fibrinogen adsorption phenomenon on the studied a-C:H films takes place in two stages: a fast first stage, where most of the protein is adsorbed on the surface, and a second slower stage. There are two proposed reasons for the existence of these two stages. These concern the relaxation time that is necessary for the Fib molecules, in order to find the orientation and conformation that allow other molecules to adsorb on the rest of the surface that is available; and the effect of locally charged areas on the surface of the thin films, that attract different domains of the Fib molecules with different adsorption rate.

As far as the antibacterial properties of the films is concerned, a-C:H and TiB_2 favour the *S.aureus* adhesion compared to silicon. However, the a-C:H and TiB_2 which present higher $\Gamma_{HSA} / \Gamma_{Fib}$ value favour less the *S.aureus* adhesion.

Finally, zinc oxide nanoparticles (ZnO NPs) were developed under three different temperatures: 23°C (composed by ZnO και $Zn(OH)_2$,size 20-30nm), 70°C (composed by ZnO, size 15-17nm) και 90°C (composed by ZnO, size 25-30nm) and polymeric films containing ZnO NPs in three different concentrations were developed. The NPs size plays an important role for the low concentrations, due to the increased surface to volume ratio, which results is increased production of H_2O_2. This penetrates the bacteria cell membrane and inhibits their growth and their survival. For high NPs concentrations, all the films containing ZnO NPs exhibit good antibacterial properties against *S.aureus*.