Summary

The interactions between implantable medical devices and human tissues depend upon a large number of factors, which determine their successful integration or failure. One of the main players is the serum protein layer, which forms on the surface of every biomaterials in few minutes after the contact with biological fluids. Hydrophobic and electrostatic interactions are the main driving forces of protein adsorption, heavily depending on surface properties surfaces. Topography, chemistry, surface free energy, wettability, and functional groups determine which proteins are adsorbed, how many of them and their conformation in the transient matrix: all fundamental factors for the host response to the implant. The possibility of controlling the formation of the protein layer will open new horizons for the design and development of new biomaterials. A lot of work has been done to unveil this matter, in particular by investigating simple systems, involving single protein adsorption on model cases, such as flat surfaces with a well-known chemistry. Unfortunately, adsorption mechanisms are still quite mysterious, due to the extreme complexity of protein-surface interactions, which relate not only on the properties of the single protein or the particular surface, but also on the surrounding environment (composition of the solution, pH, temperature). In addition, the surface of biomaterials is far from ideality, being rough, with complex chemistry and properties. All of these not being enough, there is a lack of suitable techniques to characterize events at the nanoscale that take place on surfaces with micro or even millimetric features.

The main idea of this PhD thesis is to investigate protein adsorption on biomaterials of clinical interest, in particular for orthopedic and dental applications, in conditions as close as possible to the physiological environment. Therefore, the focus was put on seven different substrates for bone contact, which include: pure titanium and Ti6Al4V alloy, polished and with three different chemical treatments; a silica-based bioactive glass with and without silver doping; polystyrene, as a model hydrophobic surface.

At first, the samples were thoroughly characterized, with particular attention on the topography, surface chemistry, exposed OH groups, surface energy, wettability, and ζ potential. Then, adsorption of albumin and fibronectin at near physiological concentration was investigated. The first goal was to find a set of techniques suitable for such a task. Conventional analysis, such as XPS or micro ATR-FTIR, were coupled with innovative approaches, such as solid surface ζ potential and Kelvin probe force microscopy. In the end, seven techniques have been merged to

obtain novel and interesting insight on protein-material interactions. Noticeably, surface topography has a central role in determining the amount of proteins adsorbed, both albumin and fibronectin, while protein denaturation is more related to acidic hydroxyl groups on titanium substrates and to silver on bioactive glasses. On them, the adsorption mechanism is further complicated by the glass reactivity, involving the incorporation of proteins inside the silica gel layer. Then, the competition for the surface was evaluated, exploiting sequential adsorptions and co-adsorption. Effect of proteins on titanium bioactivity was also assessed.

The second focus of this work was about the osteoimmunomodulation effect of different surface treatments on Ti6Al4V alloy. Osteogenic cells are intimately related to immune cells, and implant osseointegration cannot succeed without a proper inflammatory response. The foreign body reaction was evaluated by culturing macrophages on the titanium surfaces, measuring their viability and quantifying 27 different factors and chemokines released.