Summary

The Ph.D. work focused on characterizing protein adsorption on modified titanium surfaces and its impact on biological responses. Protein adsorption, influenced by surface properties like topography, roughness, and chemistry, is a crucial step for several biological responses after implantation. We investigated protein adsorption as a critical step of three biological responses: clotting, inflammatory response, and protection against infections. In this last case, protein adsorption was also harnessed as a surface modification technique to optimize implant outcomes. A first study examined the adsorption of fibrinogen (FB), a blood coagulation protein, on clinically relevant titanium surfaces. The surfaces were 2 Ti6Al4V, smooth (Ti64) and chemically treated (CT), and 4 Ti Gr2, smooth (Ti), chemically treated (NANCI), laser structured (Ti_L), and E-Beam structured (Ti_EB). In addition, two model surfaces, polypropylene and SiO_2, were investigated, as hydrophobic and hydrophilic models. The goal was to explore how surface features affect FB adsorption and conformation and propose hypotheses on the surface-protein interactions. According to the literature, a hydrophobic surface allows the formation of a network of FB because the adsorption occurs with central domains while the external αC domains interact with each other. On the other hand, FB forms single clusters dispersed on hydrophilic surfaces because the FB's αC domains are not available to form a network because they interact with the OH groups of the surface [1]. Starting from this original hypothesis, we verified it on various titanium surfaces exhibiting distinct properties, including wettability, charge, topography, and roughness, all of which influenced the conformation of fibrinogen (FB). The investigation of FB adsorption involved several analytical techniques: contact angle measurement, roughness and 3D reconstruction, zeta potential analysis, FESEM (Field Emission Scanning Electron Microscopy), KPFM (Kelvin Probe Force Microscopy), FTIR-ATR (Fourier Transformed Infrared Spectroscopy- Attenuated Total Reflectnce) mapping, ELISA (Enzyme-Linked Immunosorbent Assay), CLMS (Confocal Laser Microscopy), and BCA assays (Bicinchoninic acid assay). The conformation, quantification, and distribution mapping of FB were evaluated and compared with the already proven properties of each surface: increased osseointegration (CT and NANCI) or antifouling effect vs bacteria (Ti_L and Ti_EB). Different models for FB adsorption on the different titanium and model surfaces were deducted and hypothesized.

A second study focused on BSA adsorption on titanium surfaces chemically treated and functionalized with a polyphenol extract from red pomace (PPHE). This strategy aims to enhance osteointegration and minimize inflammation. Investigating BSA adsorption provided valuable insights into the effectiveness of the functionalization process. The samples were characterized with Zeta potential, XPS (X-ray photoelectron spectroscopy), contact angle, fluorescence microscopy, and FTIR-ATR mapping. The effect of PPHE functionalization on BSA adsorption was evaluated, and parameters such as the BSA amount, secondary structure, and type of bonding with the surface were compared and investigated.

The final study focused on creating an antibacterial titanium alloy surface by functionalizing it with Bovine serum albumin (BSA), and cysteine (Cys) as linkers for silver. Two molecules were used as silver linkers to evaluate the effect of different chemical bonds. The aim of the antibacterial functionalization was to add an antibacterial action without affecting the cytocompatibility of the surface. The main analyses performed were FESEM, XPS, ion release, indirect cytocompatibility test with hMSC (human Mesenchymal Stem cells), direct cytocompatibility (hMSCs), and antibacterial tests such as inhibition halo (S. Epidermidis) and biofilm evaluation (S. aureus). The consequences of the reduction of silver (Ag(0)) and the formation of Ag-S bonding due to the presence of BSA or Cys were investigated and analyzed as key points of the cytocompatibility and/or antibacterial action.

Finally, a comprehensive discussion of the connection between protein adsorption and the explored biological responses is reported and conclusions are derived.

Some side research works are described in the appendix.