Abstract

This PhD thesis combines the results from an individual PhD project part of PREMUROSA project aimed at optimizing materials to elicit targeted cellular responses and laying the groundwork for the production of PREMUROSA reference materials pivotal for the advancement of *in vitro* and *in silico* testing technologies for musculoskeletal applications. By studying a diverse array of tunable material surface properties, this work focuses on the intricate role of biomaterial surface characteristics to modulation of biological response, particularly focusing on titanium alloys and bioactive glasses.

In particular, this project will delve into understanding the role of surface of biomaterials through the characterization and production of reference materials featuring diverse tunable properties. The focus is on titanium alloys and bioactive glasses, either as bulk, micro-, and nano-powders, hierarchical scaffolds with multiscale porosity; tailored compositions obtained by doping with antimicrobial ions or by modulating the surface reactivity and surface functionalization with antimicrobial agents able to modulate the biological response.

A paramount challenge addressed in this research refers to combatting bacterial biofilm formation while concurrently facilitating bone regeneration—a critical aspect in orthopedic infection treatment. Traditional systemic antibiotic therapies often fall short due to biofilm resilience and concerns over bacterial resistance. Thus, the pursuit of antibiotic-free alternatives capable of fostering bone regeneration while preventing bacterial infection remains imperative.

Multifunctional biomaterials, such as those investigated here, hold promise in addressing these challenges.

The first part of this work centers on optimizing the surface functionalization process to immobilize the antimicrobial peptide nisin onto titanium alloy surfaces without resorting to toxic linkers and preventing the surface micro- and nanoroughness. Through systematic adjustments of process parameters such as pH value, enhanced efficacy of nisin immobilization was achieved, culminating in promising anti-microfouling activity against *Staphylococcus aureus (S.aureus)*. Notably, the chemically treated titanium condition exhibited antibacterial effect, due to a synergistic interplay and between nanotextured surfaces and nisin, underscoring its potential in bone-related applications.

In the next part, bioactive glass disc surfaces, were successfully doped with either ionic Cu^{2+} (Cu-SBA3) or Ag^{+} (Ag-SBA2) through an ion exchange process in aqueous solution. The concentration of the Cu(II)acetate ion exchange solution was optimized, with 0.001 M being chosen for further analyses. The Cu-doping of SBA3 surfaces was found to maintain their amorphous nature and enhance *in vitro* bioactivity, while also demonstrating significant antibacterial activity against *S.aureus*. While challenges regarding cytocompatibility of doped glasses with respect to human adipose stem cells (hASCs) were encountered, particularly with Cu-SBA3, strategies such as pre-incubation and indirect culture hold promise in mitigating cytotoxic effects. In addition, in the case of Ag-SBA2, cytocompatibility was significantly improved with an adsorbed layer of fibronectin protein on the glass surface before cell seeding.

The study also explores the fabrication of porous bioactive glass-ceramic scaffolds by foam replica method. Two different sintering temperatures were compared: one resulting in an amorphous scaffold (620 °C), and another one yielding a glass-ceramic one (850 °C). Both scaffolds closely resembled the 3D architecture of natural trabecular bone and exhibited high porosity of approximately 75 vol.%. However, due to its superior mechanical properties, only the glass-ceramic scaffold was the focus of further analysis. The scaffolds demonstrated high *in vitro* bioactivity and mechanical strength, and additional antibacterial properties provided by silver doping by ion exchange. The scaffold developed in this study could find a possible application as a bone substitution material with antibacterial properties without using traditional antibiotics.

Despite the remaining challenges, the findings presented in this thesis provide valuable insights into the varied biological responses induced by various biomaterial surfaces, serving as a promising basis for future research endeavors to advance multifunctional biomaterial surfaces for musculoskeletal applications.