Thesis title:

Physico-chemical cues to guide bone regeneration in calcium phosphate-based biomaterials

Abstract

Bone is well-known for its ability to self-repair much of the damage it experiences due to trauma or disease. However, when the damage is too severe such as for critical size defects or infections, their natural healing capacity is surpassed, and alternative approaches are required. Classical bone tissue engineering (BTE) strategies combine biomaterials, cells and growth factors to create living implants. The demand for technologies capable of improving the biomaterials used for treating large bone defects has been increasing. Consequently, various materials and manufacturing techniques have been explored leading to a better control of the geometric, mechanical, and biological properties associated with bone scaffolds. Calcium phosphates (CaP) stand out as an excellent scaffold material for bone regeneration due to their resemblance to natural bone and active promotion of osteogenesis. By controlling and optimizing the structure of CaP-based scaffolds at multiple scales, substantial advancements in BTE can be achieved. However, the design of CaP-based scaffolds and the tuning of their physical and chemical cues to maximize bone healing remains an open challenge.

In this thesis, we aimed to develop a combined experimental-computational framework to explore the key morphological drivers of bone regeneration in CaP-based scaffolds and to use these insights to design, manufacture and test optimized scaffolds in static and dynamic conditions *in vitro*.

Initially, we established a theoretical basis for this work by conducting a comprehensive systematic literature review and meta-analysis of the bone forming potential of CaP-based biomaterials tested in craniomaxillofacial (CMF) animal models. The analysis highlighted the influence of structural properties like chemical composition, particle size and pore size, as well as experimental factors including implantation time and animal species. This comprehensive meta-analysis quantitatively underscored the significance of key structural parameters in CaP biomaterials' bone regeneration potential.

Then, a data-driven model was constructed to serve as an optimization tool for enhancing CaP bone biomaterials. The dataset combined histomorphometrical data from seven commercially available intra-oral bone grafts, obtained from previous in-house studies, with their physico-chemical attributes analyzed in the current investigation. Partial least square regression (PLSR) modeling revealed that chemical composition and macroporosity held significant weight in the key properties related to effective bone tissue regeneration. This study not only enhanced our comprehension of how specific biomaterial properties influence the bone healing process but also furnished a robust instrument for designing bone biomaterials with more controlled and customized structures.

Next, we conducted exploratory and confirmatory experimental trials employing the key physico-chemical drivers identified in the previous studies. A first screening approach involved creating disk-shaped scaffolds constructed from hydroxyapatite (HAp), tricalcium phosphate (TCP), and biphasic calcium phosphate (BCP) materials with channels of different basic geometries and sizes. These disks

were seeded with bone marrow derived immortalized mesenchymal stem cells (hTERT-BMMSCs) and their growth under static conditions was quantified over time. These cells demonstrated curvature-based neotissue growth across all pore geometries. This observation confirmed the validity of previously developed mechanistic models for neotissue for CaP-based materials. An optimized 3D scaffold structure was predicted and produced, and *in vitro* testing demonstrated a good agreement between the predicted and observed neotissue formation in the scaffold.

Subsequently, the investigation into scaffold internal design progressed towards more complex and advanced structures. A series of 3D-printed triply periodic minimal surface (TPMS) scaffolds were designed *in silico* (with and without gradients in the microstructure) and additively manufactured in HAp. After seeding with hTERT-BMMSCs, the constructs were cultured in either static or dynamic culture conditions. For the latter a previously developed bioreactor was used, after modifying it to allow for the culture of multiple samples per run. All scaffold structures showed very good to outstanding performance in terms of cell viability, gene expression and *de novo* bone formation, with dynamic culture conditions outperforming static conditions. This study contributed to the assessment of the impact of spatial pore architecture and structural gradients on the biological functionality of 3D-structured CaP-based scaffolds.

In conclusion, this PhD thesis offers valuable insight into the contribution of physico-chemical attributes and internal design on the biological functionality of CaP-based bone biomaterials. This newfound knowledge directly contributes to designing and additively manufacturing optimized scaffolds for BTE applications.